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Differential involvement of amygdala subsystems in appetitive conditioning and drug addiction

by

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Summary

In this chapter, we review data from appetitive conditioning studies using measures of pavlovian approach behaviour and of the effects of pavlovian conditioned stimuli on instrumental behaviour, including the pavlovian-to-instrumental transfer effect and conditioned reinforcement. These studies consistently demonstrate double dissociations of function between the basolateral area and the central nucleus of the amygdala. Moreover, these data show marked parallels with data derived from studies of aversive (fear) conditioning, and are consistent with the idea that these subsystems of the amygdala use different associative representations formed during conditioning, as part of a larger limbic cortico-striatal circuit. We suggest that the basolateral amygdala is required for a conditioned stimulus to gain access to the current value of its specific unconditioned stimulus, while the central nucleus is responsible for conditioned motivational responses using a simpler stimulus-response representation. Though these systems normally operate together, they modulate ongoing behaviour in distinct ways. We illustrate this by considering the contributions of both systems to the process of drug addiction, using second-order schedules of intravenous drug self-administration.

Introduction

In this chapter, we will review data from our own and other studies which demonstrate that the amygdala is critically involved in distinct associative processes in appetitive settings and that the pattern of results indicates marked parallels with the mass of data derived from similar investigations of aversively motivated learning. Indeed, prevailing theories of amygdala function have for some years been based predominantly on the results of studies of conditioned fear (Davis, 1992a; Davis, 1992b; Fendt *et al.*, 1999; LeDoux, 1996; Maren *et al.*, 1996). Thus, the lateral nucleus of the amygdala is generally regarded as the primary, even unique, site for the convergence and association of aversive conditioned and unconditioned stimuli, thereby providing the locus of pavlovian fear conditioning (Clugnet *et al.*, 1990; Davis, 1992a; Fendt *et al.*, 1999; LeDoux *et al.*, 1990). Via its projections to the central amygdaloid nucleus, the lateral amygdaloid associative mechanism gains access to the neural controllers of autonomic, neuroendocrine and reflexive behavioural components of integrated emotional responses (Davis, 1992a; LeDoux *et al.*, 1988). This lateral-to-central flow of information is the basis of the dominant model of information processing within the amygdala, which identifies the lateral amygdala as the only locus of fear conditioning.

However, some data do not conform to this model. For example, although it has been shown that conditioned freezing may be impaired following BLA lesions, other indices suggest a persistence of conditioned fear in the same subjects (Killcross *et al.*, 1997a; Selden *et al.*, 1991; Vazdarjanova *et al.*, 1998). We have reported that pavlovian conditioned suppression can be established in rats with excitotoxic lesions of the basolateral area (lateral and basal magnocellular nuclei) of the amygdala (BLA), whereas their instrumental (voluntary) avoidance behaviour is impaired (Killcross *et al.*, 1997a). Moreover, rats with specific lesions of the central nucleus of the amygdala (CeN) show impaired pavlovian conditioned

suppression, but preserved instrumental avoidance behaviour (Killcross *et al.*, 1997a). This double dissociation of different forms of fear conditioning within the amygdala and the persistence of pavlovian fear conditioning in rats in which the BLA had been destroyed suggests a more complex pattern of functional interaction between the CeN and BLA. In particular, it must be considered that these nuclear domains of the amygdala and their associated cortical and subcortical circuitries subserve distinct aspects of emotional processing and do so in a way that does not conform to the prevailing, lateral-to-central model of information transfer within the amygdala. We will argue that this is not only the case for fear conditioning, but applies equally to appetitive conditioning as well. For example, discrete excitotoxic lesions of the BLA impair the way that stimuli endowed with positive affect can support goal-directed instrumental behaviour (conditioned reinforcement) and also impair second-order pavlovian conditioning and the revaluation of affective value in the neural representations of food rewards (Everitt *et al.*, 1999; Everitt *et al.*, 1992; Gallagher *et al.*, 1994; Holland *et al.*, 1999). Whilst lesions of the central nucleus of the CeN do not significantly impair conditioned reinforcement, they do disrupt appetitive pavlovian conditioning (Everitt *et al.*, 1999).

In fact, contemporary studies of the organisation and connections of the amygdala provide some basis for entertaining more complex forms of processing within its component nuclei. While it is clearly the case that sensory thalamic nuclei, high order sensory cortices and association cortex project richly to the lateral and basal nuclei and that these in turn project heavily onto the CeN, it is equally clear that essentially parallel high order sensory projections arrive directly within the CeN, especially its lateral sub-division (McDonald, 1998). Thus, the lateral/capsular division of the CeN may provide a gateway of sensory convergence which parallels that provided by the lateral nucleus (McDonald, 1998). The efferent projections of the BLA and CeN also provide clear evidence of segregation, as well as convergence. The CeN is well known to project to neuroendocrine and autonomic domains of the hypothalamus and brainstem, thereby providing a route for the BLA to access these sites. But the BLA has independent projections to the ventral striatum and prefrontal, especially orbitofrontal, cortex. Such connections give the BLA access to higher order response mechanisms than those provided by the downstream projections, as we have argued previously and demonstrated experimentally (Everitt *et al.*, 1999; Everitt *et al.*, 1992).

Two other features of amygdala anatomy should also be borne in mind. The first concerns the concept of the extended amygdala, whereby the central and medial nuclei of the amygdala are seen to be extended not only into the bed nucleus of the stria terminalis, but, more controversially, through the basal forebrain (the sub-commissural extended amygdala), incorporating the interstitial nucleus of the posterior limb of the anterior commissure (IPAC) to encroach upon the shell of the nucleus accumbens (NAcc) (Alheid *et al.*, 1988; De Olmos *et al.*, 1999). In this way the NAcc shell is seen as a complex mix of striatal neurons and CeN neurons, sharing with the CeN a variety of histochemical features and connections. Notable among the commonalities in the afferent connections of the central nucleus of the amygdala and the NAcc shell are a rich dopaminergic innervation arising from the VTA and also projections from the BLA (Alheid *et al.*, 1988). However, an alternative view of the CeN and its relationship to the striatum has been

put forward by (Swanson *et al.*, 1998), who suggest that the CeN is a specialized autonomic-projecting region of the striatum. The second feature concerns the marked projections from the CeN to the monoaminergic and cholinergic neurons of the isodendritic core, including to the noradrenergic locus ceruleus, the dopaminergic substantia nigra and ventral tegmental area, the serotoninergic raphé nuclei and the cholinergic nucleus basalis magnocellularis (Amaral *et al.*, 1992; Davis, 1992a; Gallagher *et al.*, 1994; Holland *et al.*, 1999; Price *et al.*, 1987). Increasingly, these connections are seen as providing a mechanism for the CeN to influence attentional, response activating, rewarding and other arousal processes mediated by these diffuse projections to the forebrain (Everitt *et al.*, 1999; Holland *et al.*, 1999)

Behavioural procedures

In the experiments to be described, we have used three procedures to assess associative mechanisms that impact on reflexive and voluntary behavioural responses. These are: (i) pavlovian approach behaviour, which provides a means of measuring the tendency of animals to approach stimuli that have acquired motivational salience through their predictive (pavlovian) association with a primary reward (Tomie *et al.*, 1989). (ii) The ability of an appetitive pavlovian conditioned stimulus (CS) to exert a direct effect on the vigour of instrumental behaviour (the so-called pavlovian-to-instrumental transfer effect) (Dickinson, 1994). (iii) Conditioned reinforcement – the process by which a predictive CS is able to support instrumental behaviour (Mackintosh, 1974). Although we have studied these processes in isolation in order to explore their neuroanatomical basis, they will usually occur in parallel in the integrated expression of behaviour by animals in naturalistic settings. We will illustrate this by reference to some of the associative mechanisms underlying drug-seeking behaviour which may be important determinants of the persistence of drug-taking habits in humans. We will suggest that dissociable amygdala subsystems involving central and basolateral parts of the amygdala operate in parallel, as well as in series, so as to subserve such parallel behavioural processes in integrated forms of behaviour.

(i) Appetitive pavlovian conditioning: autoshaping

Whilst in aversive conditioning experiments, pavlovian conditioned responses are readily elicited and quantified (e.g. freezing, startle), this is not so straightforward in appetitive settings. In the present experiments, we have focused on an 'autoshaping' task, in order to provide a relatively easily measurable appetitive pavlovian conditioned response (approach behaviour) and to minimise the contributions of other learning mechanisms to the process by which environmental stimuli are associated with primary reward, thereby gaining motivational salience (Hearst *et al.*, 1974; Tomie, 1996; Tomie *et al.*, 1989). The apparatus, procedure and theoretical basis of this task have been discussed elsewhere (Bussey *et al.*, 1997). Briefly, a visual stimulus (CS+) is presented on a VDU which is then followed by the delivery of food in a *different* spatial location, non-contingently with respect to the animal's behaviour. A second stimulus (CS-) is also presented, but never followed by delivery of food. Over training, animals develop a discriminated

conditioned response of approaching the CS predictive of food before returning to the food hopper to retrieve the primary reward. This preparatory approach behaviour is deemed to be under the control of pavlovian mechanisms as it lacks the behavioural flexibility of instrumental, goal-directed actions (Williams *et al.*, 1969) and has been described as a form of sign tracking, by which such stimuli capture attention and elicit automatic responses that are likely to bring animals into direct contact with primary goals (Hearst *et al.*, 1974).

Quite different consequences of inactivating the CeN and BLA were observed on this task (Everitt *et al.*, 1999). Bilateral, excitotoxic lesions of the BLA had no effect on the acquisition of autoshaping; lesioned subjects came progressively to approach the CS+ and eliminate their approaches to the CS-. By contrast, bilateral lesions of the CeN greatly impaired autoshaping such that lesioned animals did not increase their approaches to the CS+ (**Figure 1**). These results clearly indicate that pavlovian associations between an environmental stimulus and primary reinforcement can be established in the absence of the BLA, as we have also found for pavlovian conditioned suppression.

There is perhaps a tendency to assume that the amygdala alone is involved in associations between environmental stimuli and reinforcing events, especially in studies of aversive conditioning. However, not only is it clear that some forms of fear-motivated learning, such as aversive eye-blink conditioning, develop normally in the absence of a functioning amygdala, depending more on cerebellar circuitry (Lavond *et al.*, 1993), but also that it is increasingly possible to define a more widely dispersed neural network involved in such associative functions. For example it is well established that the anterior, but not posterior, cingulate cortex is critically important for the formation of stimulus-reinforcer associations. Indeed, we have shown previously that lesions of the anterior cingulate cortex profoundly impair the acquisition of autoshaping (Bussey *et al.*, 1997), while Gabriel and colleagues have demonstrated electrophysiologically and in lesion studies the involvement of the same area of cortex early in the course of aversive conditioning (Freeman *et al.*, 1996; Gabriel, 1990). Moreover, the integrity of specific areas of the ventral striatum also appear to be required for pavlovian approach behaviour to develop. Thus, while selective excitotoxic lesions of the NAcc shell were without effect on autoshaping, specific lesions of the NAcc core profoundly disrupted its acquisition (Parkinson *et al.*, in press-a). In addition, lesions of the pedunculopontine nucleus, a major output target of the ventral striatum, profoundly impair autoshaping (Inglis *et al.*, in press), thereby confirming the involvement of the ventral striatopallidal system in this form of conditioning.

Since our own tract-tracing experiments confirmed that the anterior cingulate cortex is a major source of projections to the NAcc core, we investigated the possible functional relationship between the two structures by making a “disconnection lesion” (a unilateral lesion of the anterior cingulate cortex and a contralateral, unilateral lesion of the NAcc core) and assessing the effects on autoshaping. The disconnection lesion disrupted autoshaping as effectively as bilateral lesions of either structure alone (Parkinson *et al.*, in press-a). This provides strong evidence for a functional connection between the anterior cingulate cortex and NAcc core, but leaves open the issue concerning the relationship between the CeN and

this limbic corticostriatal circuit that underlies appetitive pavlovian conditioning. This is a critical issue because our data together indicate that each of these structures is necessary, but not sufficient, for autoshaping to develop. Since there is no obvious direct connection between the CeN and this cingulate cortex-NAcc core circuit, the answer may involve regulation of the dopaminergic innervation of the NAcc by the CeN.

There are substantial projections from the CeN to the VTA and substantia nigra that we have recently confirmed by anterograde and retrograde tract-tracing studies (Hall, Parkinson and Everitt, unpublished observations). It has been shown in very different kinds of study that manipulations of the CeN can influence dorsal and ventral striatal dopamine function. For example, dopaminergic lesions of the CeN or infusions of dopamine receptor antagonists into the amygdala both affect levels of extracellular dopamine in the NAcc and also cocaine self-administration (which depends upon the integrity of the mesolimbic dopamine system, (Caine *et al.*, 1995; Hurd *et al.*, 1997; Louilot *et al.*, 1985; McGregor *et al.*, 1993; Simon *et al.*, 1988). Moreover, a disconnection lesion of the CeN and the dopaminergic innervation of the dorsolateral striatum has been shown to impair the acquisition of a conditioned orienting response (Han *et al.*, 1997). Consistent with this notion of a link between the CeN and the mesolimbic dopamine system, we have shown in our recent studies (Parkinson, Bamford, Fehnert, Dalley, Robbins and Everitt, 1999) that 6-hydroxydopamine-induced dopamine depletion from the NAcc abolishes the acquisition of autoshaping.

Taken together, these results suggest a distributed neural network underlying pavlovian approach behaviour that involves the anterior cingulate cortex, NAcc core, CeN and mesolimbic dopamine system (**Figure 2**). The anterior cingulate cortex may be of primary importance in this network, projecting as it does both to the NAcc core and also to the CeN, the latter projections having been suggested to be the route via which the anterior cingulate cortex influences autonomic responses (Vogt, 1985). The elements of this network have complementary patterns of connectivity that allow them to mediate the component processes underlying an integrated response to appetitive stimuli. Thus, we hypothesize that the anterior cingulate cortex-NAcc core system may mediate pavlovian associative processes and give direction to pavlovian approach responses (Everitt *et al.*, 1999; Parkinson *et al.*, in press-a). But clearly, information about appetitive stimuli also impinges on the CeN from a variety of sources (including the anterior cingulate cortex, high-order sensory cortices, BLA and thalamus), so enabling the CeN, through its differentiated outputs, to orchestrate not only autonomic and endocrine responses but also different forms of arousal processes dependent upon the chemically-defined systems of the reticular core of the brain (Davis, 1992a; Everitt *et al.*, 1999). Projections from the CeN to the mesolimbic dopaminergic neurons in the VTA, for example, may regulate behavioural activation, thereby invigorating approach responses, while also enhancing the coupling of the anterior cingulate cortex-NAcc core circuit that provides direction to those responses (Everitt *et al.*, 1999). In addition, and perhaps especially early in conditioning, the CeN will engage attentional mechanisms through interactions with the basal forebrain cholinergic system (Han *et al.*, in press), and also orienting responses through interactions with the nigro-striatal dopaminergic system innervating the dorsolateral caudate-putamen (Han *et al.*, 1997).

A clearer understanding of these processes requires experiments designed to explore the specificity of the effects of manipulations of each of the components of this network on the development of the pavlovian approach response. For example, it is uncertain whether impairments in autoshaping reflect disruption of the associative process *per se* or the orchestration and coupling of response mechanisms. Prevailing data would implicate the anterior cingulate cortex in the former and the NAcc core with its dopaminergic innervation in the latter. But the CeN may have much more complex functions that transcend associative, output, attentional and arousal processes. Traditionally, functions of the CeN have been constrained by its perceived role as simply subordinate to the lateral and basal nuclei of the amygdala and its projections to neuroendocrine, autonomic and primitive motor domains of the hypothalamus and brainstem (Fendt *et al.*, 1999; LeDoux, 1996). But increasingly, more diverse afferents to the CeN have been demonstrated (McDonald, 1998), while its efferents to the neurochemically-defined diffuse projection systems of the isodendritic core (arousal systems) have to be taken into account in order to understand the co-ordinating and integrative functions of this component of the amygdaloid nuclear complex. These latter projections bring the entire forebrain under the modulatory control of the CeN (**Figure 3**).

(ii) Pavlovian-to-instrumental transfer

The key demonstration of the motivational impact of pavlovian CSs on instrumental behaviour comes from an experiment by (Lovibond, 1983). Rabbits were trained to lift a lever for delivery of a sugar solution directly into the mouth. The lever was then removed in a second, pavlovian stage in which a 10-second stimulus was repeatedly paired with the sugar solution. In the final stage, presenting the pavlovian CS while the animal was performing the instrumental task resulted in a marked elevation of responding – an effect of the CS which is also modulated by the animal's motivational state. While there has been some debate about the precise mechanisms underlying this so-called pavlovian-to-instrumental transfer effect (Dickinson, 1994), it is generally assumed that the pavlovian CS exerts a general motivational, or activational influence on goal-directed instrumental behaviour. The procedure we have used in the experiment reported here was essentially that described by (Balleine, 1994). Briefly, animals were first trained to associate presentations of a 2-minute auditory stimulus (CS) with delivery of food pellets into a hopper. During training, animals came to approach the food hopper preferentially during the CS period. After this pavlovian conditioning, the animals were trained to lever press for the same food reward in the absence of the CS. Finally, in the test phase, the ability of the CS to enhance lever pressing in extinction was assessed, compared to equivalent periods of both baseline responding and responding to a control stimulus (one explicitly not paired with food).

Bilateral excitotoxic lesions of the BLA had no effect on this pavlovian-to-instrumental transfer procedure. However, selective bilateral lesions of the CeN blocked completely the ability of the CS to enhance lever pressing in the test phase (**Figure 4**, Hall, Parkinson, Connor, Dickinson and Everitt, unpublished). This result directly parallels the dissociation of effects of CeN and BLA lesions on pavlovian

approach behaviour and leads us to speculate that the neural basis of this effect of the CS to invigorate instrumental behaviour again depends upon interactions between the CeN and the mesolimbic dopamine system. Some evidence in support of this speculation comes from the observation that the dopamine receptor antagonist, pimozide, also blocks the pavlovian-to-instrumental transfer effect, thereby indicating a dopaminergic mechanism mediating this motivational influence of a pavlovian CS (Smith *et al.*, 1998).

(iii) Conditioned reinforcement

Appetitive pavlovian CSs not only elicit behavioural arousal and approach responses, but by acquiring some of the properties of a goal they gain motivational salience and thereby control over instrumental - or voluntary - behaviour as conditioned reinforcers (Everitt *et al.*, 1992; Mackintosh, 1974; Robbins, 1978). We have studied this using a procedure that isolates the conditioned reinforcement process. Briefly, there are two phases to the procedure. First, rather as in the autoshaping procedure, a neutral stimulus (light, sound or a compound of both) is paired with primary reward (we have used water in thirsty subjects, sucrose in hungry subjects or intravenous cocaine) and the development of pavlovian conditioning is assessed by measuring discriminated approach to the CS+. In the second phase, which is carried out in extinction, thereby removing any influence of primary reinforcement, two novel levers enter the testing chamber; responding on one of them (CRf lever) results in presentation of the light CS+. Responding on the second lever (NCRf lever) has no programmed consequence. The acquired motivational properties of the CS to serve as a conditioned reinforcer is therefore assessed by its ability to reinforce the acquisition of this novel and arbitrary response. An important aspect of this process is that the control over behaviour by a conditioned reinforcer (CRf) is powerfully amplified by psychomotor stimulants and this effect has been shown to depend critically upon the dopaminergic innervation of the NAcc (Robbins *et al.*, 1989; Taylor *et al.*, 1984; Taylor *et al.*, 1986). However, even in the face of extensive dopamine depletion from the NAcc, or general dopamine receptor blockade, rats still acquire a new response with conditioned reinforcement (Robbins *et al.*, 1989; Taylor *et al.*, 1986; Wolterink *et al.*, 1993). That is, the mesolimbic dopamine system does not mediate conditioned reinforcement, but only its potentiation by stimulant drugs. Thus, information about conditioned reinforcers must be derived from another source, presumably one transferring such information to the NAcc where its impact can be gain-amplified by increases in dopamine transmission (Robbins *et al.*, 1989; (Everitt *et al.*, 1992). A wealth of neuroanatomical data emphasizes the limbic cortices as the primary sources of information processed within the NAcc, most notably the BLA, hippocampal formation (via the subiculum), prelimbic and anterior cingulate cortices (De Olmos *et al.*, 1999; Groenewegen *et al.*, 1996; Kelley *et al.*, 1982a; Kelley *et al.*, 1982b; Mogenson *et al.*, 1984). We reviewed much of our data on the differential contributions of these sources of afferents to the NAcc on conditioned reinforcement and its dopaminergic modulation in the previous edition of this volume (Everitt *et al.*, 1992). These data will be briefly summarised here and more recent findings emphasized.

As can be seen in **Figure 1**, rats with selective BLA lesions are impaired in their acquisition of a new response, failing to respond selectively upon the CRf lever (Burns *et al.*, 1993; Cador *et al.*, 1989). Thus

although BLA lesions were without major effects on appetitive pavlovian conditioning (see above), the control over behaviour by the CS was attenuated and, as a consequence, 'gain amplifying' effects on conditioned reinforcement of intra-NAcc infusions of d-amphetamine were also greatly reduced. These results are consistent with a burgeoning literature that shows marked effects of BLA lesions on the control over instrumental behaviour by pavlovian CSs using a variety of behavioural procedures, including so-called conditioned place - or cue - preference, a superficially simple task that in fact embodies both pavlovian approach behaviour and instrumental response contingencies (Everitt *et al.*, 1989; Everitt *et al.*, 1991; Hiroi *et al.*, 1991).

By contrast, lesions of the ventral subiculum, a major outflow structure of the hippocampal formation, did not affect conditioned reinforcement itself (lesioned subjects still responded selectively on the CRf lever), but the potentiative effects of intra-accumbens amphetamine on the control over behaviour by the conditioned reinforcer *were* abolished, in parallel with the locomotor stimulant effects of amphetamine (Burns *et al.*, 1993). Neither prelimbic nor anterior cingulate cortex lesions had any effect on responding with conditioned reinforcement (Burns *et al.*, 1993) (Cardinal, Robbins & Everitt, unpublished).

Thus, while the BLA and ventral subiculum sources of afferents to the NAcc are both essential for conditioned reinforcement and its potentiation by increased activity in the mesolimbic dopamine system, their involvements are dissociable: (i) the BLA is part of the mechanism whereby pavlovian CSs control instrumental behaviour as conditioned reinforcers and provides the substrate for the potentiative effects of stimulant drugs on conditioned reinforcement that are expressed in the NAcc; (ii) the ventral subicular outflow from the hippocampal formation to the NAcc is essential for the potentiation of locomotor activity and conditioned reinforcement by stimulant drugs, but does not mediate informational aspects of the conditioned reinforcement process itself. We have hypothesized that the contribution of ventral subicular processes to this potentiation may be to provide the contextual background upon which the enhancement of locomotor activity presumably depends (Burns *et al.*, 1993; Everitt *et al.*, 1999). Additionally, psychomotor stimulation provides a mechanism by which the effects of conditioned reinforcers are 'gain-amplified' (**Figure 5**).

More recently, we have assessed the consequences of manipulating the CeN on conditioned reinforcement and its potentiation by increased NAcc dopamine. Excitotoxic lesions of the CeN did not impair responding with conditioned reinforcement (**Figure 1**) but completely abolished the effects of intra-NAcc infusions of d-amphetamine (Robledo *et al.*, 1996). This again demonstrates an intimate relationship between the CeN and the mesolimbic dopaminergic innervation of the NAcc, in this case specifically the shell (see below), so far as the potentiation by stimulants of responding with conditioned reinforcement is concerned. Thus, selective excitotoxic lesions of the shell abolished the effects of intra-accumbens amphetamine (and significantly attenuated the locomotor stimulant effects of systemic amphetamine), but did not interfere with the control over instrumental behaviour by the conditioned reinforcer (Parkinson *et al.*, 1999). These effects of shell lesions are similar to those of ventral subiculum lesions, an observation that

is of interest in the context of the strong preferential glutamatergic projection from the ventral subiculum to that part of the NAcc shell (septal pole) which was lesioned in these experiments (Kelley *et al.*, 1982a).

The effects of excitotoxic NAcc core lesions were more complex. First, unlike shell lesions, core lesions retarded the re-attainment of criterion levels of pavlovian discriminated approach that preceded the acquisition of a new response (Parkinson *et al.*, 1999). These data suggest that not only is the NAcc core involved in learning, but also in the expression, of the discriminated approach response – perhaps indicating a difference in NAcc core and amygdala involvement in this behaviour. Second, although NAcc core lesions did not significantly affect the acquisition of responding with conditioned reinforcement under saline conditions, the interaction between intra-NAcc d-amphetamine and responding with conditioned reinforcement was affected by lesions of the NAcc core, in that there was a loss of selectivity in the potentiation of responding (Parkinson *et al.*, 1999). Subjects with lesions of the BLA showed similar impairments, including a loss of control over responding for the CRf under control conditions (Burns *et al.*, 1993). We have reported previously similar effects of manipulations of the NAcc and BLA, leading us to suggest that the integrity of the BLA is critical for stimulus-reward information to gain influence over voluntary behaviour (Everitt *et al.*, 1999; Everitt *et al.*, 1992). Thus, information reaching the NAcc concerned with the nature and direction of behaviour depends upon the BLA, presumably via its projections to both the core and shell, and may be 'gain amplified' by dopamine transmission in the shell in a way that is critically dependent on its glutamatergic inputs arising from the ventral subiculum (**Figure 5**).

Furthermore, in animals with NAcc shell lesions and an intact NAcc core, we observed that intra-NAcc infusions of d-amphetamine dose-dependently increased magazine approach during the acquisition of a new response with conditioned reinforcement, suggesting that the dopaminergic innervation of the NAcc core may also modulate the vigour of pavlovian responses (Parkinson *et al.*, 1999).

It is intriguing that both the CeN and NAcc shell – often considered as a functional continuum, the "extended amygdala", mediate the important property of psychostimulant drugs to potentiate the impact of conditioned reinforcers. Consistent with our arguments above, we consider that the CeN (via its projections to the ventral tegmental area) regulates the dopaminergic system innervating the NAcc shell, mediating stimulant drug effects on instrumental behaviour and locomotor activity, and the NAcc core, mediating effects on pavlovian approach behaviour. It is difficult to postulate another mechanism by which damage to the CeN can so effectively prevent the conditioned reinforcement potentiation that follows intra-accumbens infusions of d-amphetamine (Everitt *et al.*, 1999).

Previously, when discussing interactions between the amygdala and ventral striatum, our attention has been focused on glutamatergic projections from the BLA directly to the NAcc core and shell and dopaminergic modulation of this cortico-striatal loop at that site (Everitt *et al.*, 1999; Everitt *et al.*, 1992) – much as envisaged by Mogenson (Mogenson *et al.*, 1984). But it is now clear that interactions between amygdala and NAcc are more complex in that the CeN is also able to modulate ventral and dorsal striatal

processing, not via direct projections, but by influencing the activity of the mesolimbic and nigrostriatal dopaminergic pathways. Moreover, dopaminergic mechanisms within the CeN and BLA also influence sub-components of pavlovian conditioning in a dissociable way. Thus, pre-trial infusions of 7-OHDPAT into the BLA, but not the CeN, impaired acquisition of a new instrumental response with conditioned reinforcement, whereas pavlovian approach behaviour, measured in the same task, was impaired by CeN, but not BLA, infusions of the drug (Hitchcott *et al.*, 1997a; Hitchcott *et al.*, 1998). These dissociations in associative functions of sub-regions of the amygdala following dopaminergic manipulations support the evidence and hypotheses derived from lesion studies reviewed above.

Synthesis

Dissociable processes within the amygdala

The results of our experiments summarized so far emphasize the importance of the amygdala in associative processes engaged during appetitive behaviour. Although initial studies of amygdala function, involving gross aspiration techniques that destroyed much of the temporal lobe, also revealed effects in appetitive settings (Gaffan, 1992; Henke *et al.*, 1972; Kluver *et al.*, 1939; Weiskrantz, 1956), more recent studies of the amygdala both in animals and in humans have been dominated by fear conditioning, perhaps at the cost of minimising the more general importance of this collection of temporal lobe nuclei in associative processes. Indeed, our recent data indicate marked parallels in the effects of CeN and BLA lesions on both appetitive and aversive conditioning. Thus, lesions of the CeN impair both appetitive pavlovian approach responses and pavlovian conditioned suppression, whereas BLA lesions were without effect on these measures. By contrast, BLA lesions have been shown to impair the acquisition of instrumental, or voluntary, responses with both positive and negative conditioned reinforcement, whereas CeN lesions were without effect. These dissociations of CeN and BLA function have also clearly been demonstrated by Gallagher, Holland and co-workers in appetitive paradigms by studying the effects of selective excitotoxic lesions of these subdivisions of the amygdala (Gallagher *et al.*, 1990; Gallagher *et al.*, 1992; Holland *et al.*, 1999), but this distinctive pattern of results does not only depend on the use of lesions. For example, as indicated above, Phillips and co-workers have demonstrated striking differences in the effects of dopaminergic manipulations of the BLA and CeN on instrumental and pavlovian conditioned responses, respectively (Hitchcott *et al.*, 1997a; Hitchcott *et al.*, 1997b; Hitchcott *et al.*, 1998).

Clearly, these sets of data do not sit comfortably with the notion that associations are formed and stored exclusively within the BLA and that this processing is an antecedent of subsequent response production orchestrated by the CeN. Subjects with lesions or other forms of inactivation of the BLA should not, according to this scheme, be able to form associations between environmental stimuli and reinforcing events and hence they should be unable subsequently to mount adaptive behavioural responses in the presence of such predictive cues. The explanations for these discrepancies in the data, if they are indeed discrepancies, should be considered.

One possibility that has been suggested to explain the preservation of some forms of fear conditioning in rats with BLA lesions is that the lesions themselves are 'not complete' (see Davis, this volume). Excitotoxic lesions of the BLA such as we have made are never 'complete' in every subject, as lesion schematics make clear by showing the smallest and largest lesions (e.g. Killcross *et al.*, 1997). But in many subjects we have seen no neuronal survival within the lateral and basal magnocellular nuclei at any anterior-posterior level and such subjects showed preserved classical fear conditioning. This total neuronal loss from the BLA has been confirmed in a recent study (Hall and Everitt, unpublished observations) in which the neuron-specific antibody n-Neun was used to demonstrate cell loss immunocytochemically: following quinolinic acid-induced BLA lesions, no BLA neurons survived in the lateral and basal magnocellular nuclei. But to dwell on this point is also to miss the wider importance and interpretation of the *double dissociation* of the effects of CeN and BLA lesions: pavlovian conditioned suppression *was preserved* in BLA lesioned rats that showed marked impairments in conditioned punishment. Even if a tiny population of neurons were preserved in the BLA (which in many cases they were not), this is a dramatic result. It should also be emphasized that in interpreting these effects we have never asserted either (i) that serial information transfer from lateral to central amygdala does not underlie fear conditioning - clearly it does (e.g. freezing and fear-potentiated startle) - or (ii) that the CeN is a site of CS-US *association*; whether it is or not remains to be shown, but the CeN is clearly important for some forms of fear conditioning to occur. We return to a possibly more interesting resolution of these issues below.

A second possibility that we have raised previously concerns differences in the conditioning procedures used. In the great majority of conditioned fear paradigms, very few (from one to five) pairings of CS and shock are used, whereas in our own fear conditioning studies (Killcross *et al.*, 1997a), there were many pairings of CS and US. Similarly, in the autoshaping procedure, there were many pairings of ingestive reward and the CS. This has led to the suggestion that extended training somehow recruits otherwise redundant neural mechanisms to support learning – a suggestion based in part upon the assumption that small numbers of CS-US pairings represent the critical test of fear conditioning (Fendt *et al.*, 1999; Nader *et al.*, 1997). While it is clearly the case that such associations can and do form in one trial and that it is adaptive to learn the value of danger signals rapidly, repeated pairings of CS and US, whether appetitive or aversive, are very likely to occur in naturalistic circumstances and learning in these circumstances is in itself adaptive, allowing initial associations to be modified by subsequent experience. We find it difficult to accept the notion that pavlovian associations formed over multiple trials recruit otherwise redundant neural mechanisms (Killcross *et al.*, 1997b).

Another issue of significance in this debate concerns the nature of the measures of conditioned fear. As has been argued elsewhere (Cahill *et al.*, 1999; Killcross *et al.*, 1997a), it is important to distinguish the effects of a neural manipulation, such as a lesion, on mnemonic versus non-mnemonic processes – perhaps especially the preserved ability of a subject to make the response that subsequently provides the measure of conditioning. In the case of conditioned freezing or fear-potentiated startle, there are few, if any,

demonstrations that BLA manipulations impair the conditioned responses of freezing or startle without also affecting unconditioned freezing or startle. Indeed, Davis and colleagues have provided evidence that the BLA is critical for unconditioned freezing and fear potentiated startle (Davis, 1997; Walker *et al.*, 1997). Although we have shown recently that rats with excitotoxic lesions of the BLA can, with over-training, develop a freezing response to contextual cues (Hall and Everitt, to be published), this result violates the generally held rule that rats cannot acquire conditioned fear responses to contextual cues without an intact BLA, a belief held even though we have previously and clearly demonstrated contextual fear in BLA-, but not in hippocampal-, lesioned rats (Selden *et al.*, 1991).

Inherent in the debate about associative processes in the amygdala is the notion that associations are formed exclusively in the lateral amygdala via the convergent activation of pathways conveying information about the CS and US (Clugnet *et al.*, 1990; Maren, 1996; McKernan *et al.*, 1997). But it is also clear that structures extraneous to the amygdala also participate in pavlovian associative processes, including the anterior cingulate cortex, the NAcc core and the hippocampus itself (Bussey *et al.*, 1997; Cahill *et al.*, 1999; Gabriel *et al.*, 1991; Maren *et al.*, 1997; Parkinson *et al.*, in press-b). These data derive from lesion and electrophysiological approaches and include aversive and appetitive tasks. Moreover, the lateral capsular division of the CeN is also in receipt of thalamic, cortical and subcortical afferents, suggesting that the ingredients of such associative learning exist within this component of the amygdala (McDonald, 1998), even though associative long-term potentiation (LTP) has not to date been demonstrated there as it has within the lateral nucleus (Clugnet *et al.*, 1990; Maren, 1996; McKernan *et al.*, 1997). However, neurons in the CeN do respond to both conditioned and unconditioned fear stimuli (Pascoe *et al.*, 1985b) and undergo plastic changes during fear conditioning (Applegate *et al.*, 1982; Pascoe *et al.*, 1985a). It should also be noted that there is also evidence of plasticity within structures afferent to the amygdala, most notably in the thalamic medial geniculate nucleus during auditory fear conditioning (Cahill *et al.*, 1999).

What representations are formed during conditioning?

It is timely to consider the existing results at face value and consider what explanations can best accommodate the main body of data. The lack of effect of BLA lesions on pavlovian conditioning in some of our own, as well as in other, studies is perhaps at first sight surprising, as there are many demonstrations, again including our own, that BLA lesions can impair aversive pavlovian conditioning. For example, the integrity of the BLA has been shown to be critical for the acquisition of appetitive conditioned place preferences as well as place aversions (Everitt *et al.*, 1991; Hiroi *et al.*, 1991; Selden *et al.*, 1991). It must be appreciated that although conditioned place preferences and aversions provide useful measures of associations between environmental stimuli and reinforcement, the nature of the precise learning mechanisms operating during their acquisition and performance is ambiguous, being consistent with the involvement of the BLA in either pavlovian or instrumental behaviour (Everitt *et al.*, 1992; McAlonan *et al.*, 1993; McDonald *et al.*, 1993). However, when considering pavlovian procedures unconfounded by conditioned instrumental performance, as above, it is increasingly clear that the BLA is not always critical

for this form of learning. Thus, results with the autoshaping paradigm used in the studies summarized here, as well as those assessing conditioned suppression, indicate a general role for the CeN in pavlovian conditioned responding. But when considering conditioned freezing and fear-potentiated startle, both BLA and CeN lesions are effective in disrupting conditioning.

Perhaps one way of disentangling these issues is to consider the nature of the representations underlying pavlovian conditioning, and associative learning in general. Associations are generally believed to be represented in the brain by altering the 'weights' of unidirectional synapses. As synaptic weights can only change on the basis of information available to the neurons involved, the association of representations A and B in **Figure 6** can only occur at points where information about these two representations converge, no matter what mechanisms exist to supervise and use the association. Such associations may be used for different purposes – for example, as a representation of a higher-order property of stimuli (a "feature detector"), or for commanding behavioural responses directly.

In pavlovian conditioning, there is the potential for several associations to form, as illustrated in **Figure 7**. Lesions that remove a representation of either the CS, the US or the response would have obvious consequences not only for conditioned, but also unconditioned, responding (sites A, B, D in the figure). Lesions of site C, representing a central motivational state (such as fear) might not impair primitive unconditioned responses, yet could impair conditioned responses that were based on the elicitation of fear. Again, however, any properties of the unconditioned response to the US that depended on this hypothesized "fear" state would be lost.

Experimental analysis of pavlovian conditioning has shown that CS-US pairings may cause the CS to elicit at least three of these representations in the brain (Gewirtz *et al.*, 1998; Mackintosh, 1983). The first and simplest of these is that the CS may become directly associated with the *unconditioned response* (UR), forming a simple stimulus-response association.

The second is a representation of *affect*, as demonstrated by the phenomenon of transreinforcer blocking, in which a CS paired with shock can block conditioning to a CS paired with the absence of otherwise expected food reward (Dickinson *et al.*, 1979). These two reinforcers share no common properties other than their aversiveness and therefore the blocking effect must depend upon an association between the CS and affect. Affective states can therefore be independent of the specific reinforcer and response. This concept has been widely used in theories of learning (Dickinson *et al.*, 1979; Konorski, 1948; Konorski, 1967) and is illustrated in **Figure 8**. Associations between the stimulus and an affective state appear to be critical in second-order conditioning (S_1-US followed by S_2-S_1); unlike a first-order conditioned response (CR), a second-order CR is relatively insensitive to post-training changes in the value of the US (implying that it does not depend on S_2-US associations) and the response to S_2 may differ from the response to S_1 or the US (implying that it does not depend on S_2-R associations).

The third form of representation is *specific to the US*. If a CS is paired with a desirable food and the food is subsequently devalued by pairing it with LiCl, such that the food now becomes aversive and elicits appropriate responses, the reaction to the first-order CS changes in normal animals (Mackintosh, 1983). Therefore the CS cannot have been associated with an abstract "positive affect" representation, but must have been associated with that particular reinforcer. The association must be specific to the US because the reaction to a second CS that predicted a different food does not alter, and its connections with valence information must be modifiable and *downstream* of the CS-US association.

Further evidence that a CS becomes associated with a relatively specific representation of the properties of a reinforcer is provided by studies of cued instrumental discrimination (Trapold, 1970). Rats acquired an instrumental discrimination between two levers paired with different appetitive reinforcers more rapidly if the discriminative cues had been paired with the same reinforcers (same condition) in a previous pavlovian stage than when the outcome was switched between stages (different condition). A rigorous demonstration of the formation of associations between the *specific sensory properties* of stimuli comes from sensory preconditioning, the process by which neutral stimuli are paired in the form S_2-S_1, after which S_1-US conditioning causes a CR to occur to S_2. Thus, in the first stage, associations form between representations that have no motivational component. Taken together these procedures demonstrate that animals are able to encode the relationship between a CS and specific sensory properties of the US and furthermore that they can relate this sensory representation to the affective valence of the reinforcer.

Nature of representations in different regions of the amygdala

Basolateral amygdala: A great deal of evidence has accumulated showing that rats with BLA lesions can acquire first-order conditioned responses, but that these responses are insensitive to reinforcer revaluation. For example, rats with BLA lesions acquired normal food cup responding to a CS paired with food, and showed normal acquisition of an aversion to that food when it was subsequently paired with LiCl, but failed spontaneously to adjust their responding (orienting and food cup approach) to the CS after the food was devalued (Hatfield *et al.*, 1996). The most parsimonious explanation is that the conditioned responses learned by these rats were a result of direct associations between the CS and the response. They lacked the ability to use the CS to access the value of a specific US and use that representation to alter their response. Holland and Gallagher define this ability as "mediated performance": the ability to respond based on a CS-activated representation of the US (Holland *et al.*, 1999).

The idea that BLA-lesioned animals cannot use a CS to gain access to the current value of its specific US has great explanatory power. Second-order conditioning requires that the second-order stimulus becomes associated with the affective value that is called up by the first-order CS (as discussed above); BLA-lesioned rats cannot acquire second-order conditioning (Hatfield *et al.*, 1996), cannot acquire

responding under second-order instrumental schedules (Everitt *et al.*, 1989; Whitelaw *et al.*, 1996), or use a first-order CS as a conditioned reinforcer (Burns *et al.*, 1993; Cador *et al.*, 1989) (see Table 1). Clearly, the responses which still occur to the first-order CS do not support second-order conditioning, while the effects on reward devaluation demonstrate that the deficit in BLA-lesioned animals is not restricted to second-order conditioning *per se*. Specific modulation of instrumental choice behaviour by a CS also requires that the subject utilizes the motivational value of a particular US; in this capability, too, BLA-lesioned animals are impaired (Killcross, 1998; Killcross *et al.*, 1997a).

The formation of an association between a CS and the affective value of a US also accounts for responses such as conditioned freezing, which cannot readily be accounted for in terms of a CS-UR association. Thus, the conditioned freezing response does not resemble the UR to shock, which is characterized by agitation, jumping, vocalization and escape, but instead represents an adaptive response to danger. At the time of conditioning, therefore, there is no freezing response occurring to which a CS-UR association can be formed. In addition, freezing is a US-specific conditioned response: while freezing occurs to a CS for shock, it does not occur to a CS for the omission of expected food, even though both signal aversive events (as discussed above). It seems plausible to suggest, therefore, that the BLA is critical for the acquisition of conditioned freezing because it subserves the formation of an association between the CS and a neural representation of the affective properties of the US (Bolles *et al.*, 1980). Similarly, fear-potentiated startle may reflect the potentiation of a reflexive startle response by an affective representation retrieved by the CS, and is thereby sensitive to BLA lesions.

Central nucleus: Even though it receives neuronal afferents appropriate to support them, there is no direct evidence to suggest that the CeN is itself a site of CS-US associations; it might receive an already-associated input. However, it is clear from the data discussed above that animals lacking a BLA can form some kinds of association, the conditioned expression of which is sensitive to CeN, but not BLA, lesions. The simplest analysis at present seems to be that the CeN does form simple CS-UR ('sensorimotor') associations, which do not depend upon a specific US: that is, they are independent of the identity and current motivational value of the US and are also unable to support second-order conditioning. We suggest that the responses subserved by CeN-dependent associations especially include the modulation of reflexes organized within the brainstem, including some that might conventionally be regarded as 'affective', including conditioned suppression, conditioned orienting and pavlovian-instrumental transfer. These are all disrupted by CeN lesions (see Table 1). Responses such as conditioned suppression may influence instrumental behaviour non-specifically, but are insufficient to modulate instrumental behaviour differentially, as assessed in choice tasks (Killcross *et al.*, 1997a). This is not to deny their role in discriminated approach as assessed by autoshaping. Indeed, this is a capability that the BLA does not seem to contribute to (Everitt *et al.*, 1999).

This view of amygdala function is illustrated speculatively in **Figure 9**. When a CS predicts an appetitive US, it may form associations with sensory and motivational representations of that US (links 1

and 2), with central affective states (3) and with unconditioned responses at some level (4). When the US is devalued, its motivational representation is in some way selectively redirected to an aversive state (not shown), so it is through link 1 or 2 that the changed response to a first-order CS occurs. It should be noted that while affective states are illustrated as "centres", very little is known of the neuronal mechanism by which valence might be encoded: such information might just as easily be carried as a temporal or chemical code and be multiply represented, rather than existing in distinct spatial loci. Indeed, it has been convincingly argued that the orbitofrontal cortex provides an important site for the representation of affective valence (Rolls, 1999; Schoenbaum *et al.*, 1998; Schoenbaum *et al.*, 1999).

Unresolved issues

It is at present unclear whether the BLA is involved in representing specific sensory information about USs, required for S–S associations. Each sensory modality projects to a region of sensory cortex, a reason to question *a priori* whether the BLA is required, and rats can learn stimulus discrimination tasks in the absence of the BLA (Burns *et al.*, 1999; Sarter *et al.*, 1985; Schwartzbaum, 1965). If the BLA is involved, it would therefore have to be as an "independent associator" (E in **Figure 7**). According to this scenario, BLA-lesioned animals make unconditioned responses and learn simple CS-UR associations, including "emotional" responses, but the CS would convey no information about the identity of the US. Alternatively, the US-specific representation involving the BLA might be purely affective; in this alternative scenario, BLA-lesioned animals can learn CS-UR associations and CS-US(sensory) associations, but cannot learn CS-US(affective) associations, and the sensory representation they can activate is without affective valence. One crucial test will be to see if BLA lesions impair sensory preconditioning.

Similarly, it is unclear whether the BLA holds US-specific representations which excite general appetitive / aversive states in another structure, or itself contains this "affective processor", or contains both. It is clearly difficult to distinguish whether BLA-lesioned animals lack affective states that may take part in associations, or merely cannot call them up via a CS; however, transreinforcer blocking and performance (but not acquisition) of second-order conditioning are two phenomena that appear to depend on simple affect.

In summary, it remains uncertain whether the BLA (i) encodes the specific sensory properties of a US, (ii) encodes the affective nature of a US, or is (iii) required for coupling *both* the sensory and affective properties of a US. Therefore, determining the subtleties of processing subserved by the BLA is important and will require further experiments.

Dissociable amygdala subsystems

The dissociations demonstrated in our studies between CeN and BLA function may reflect the more recent evolutionary development of lateral and basal nuclei of the amygdaloid complex and the ability of

the BLA to modify more primitive processes organized within the CeN (Swanson *et al.*, 1998). Although sharing some afferent connections, the BLA and CeN also show marked differences both in terms of their afferents, and also especially in their efferent connections. In particular, the distributed outputs of the CeN allow it to orchestrate autonomic, endocrine and behavioural responses that characterize integrated patterns of emotional behaviour (Davis, 1992a; Krettek *et al.*, 1978; LeDoux *et al.*, 1988; Swanson *et al.*, 1981; Zahm *et al.*, 1999). These are most frequently measured in studies of fear conditioning in terms of increased heart rate, increased adrenal hormone secretion and responses such as freezing or startle (Davis, 1992a; LeDoux *et al.*, 1988), but they are evident in appetitive situations as well and include not only approach and orientation responses, but also ingestive reflexes such as salivation and possibly other responses of the cephalic phase (Lagowska *et al.*, 1975; Roozendaal *et al.*, 1990). These responses orchestrated by the CeN depend upon downstream projections primarily to the hypothalamus and brainstem (Swanson *et al.*, 1981). The phylogenetically more recently developed BLA can recruit this response output mechanism via its rich projections to the CeN. But in addition, via its unique projections to the orbital prefrontal cortex and ventral striatum (Amaral *et al.*, 1992), the BLA may also influence more flexible responses, including goal-directed instrumental behaviour, perhaps at the same time overriding reflexive responses engendered by simple CS-UR mechanisms within the CeN.

We have also highlighted here what we consider to be a key aspect of CeN function, which is to influence ascending arousal systems through its projections to the brainstem monoaminergic and basal forebrain cholinergic neurons of the isodendritic core. These connections may not only influence attentional and plastic processes through cholinergic projections to the cortex, as demonstrated in elegant experiments by Gallagher and Holland (Gallagher *et al.*, 1994; Holland *et al.*, 1999), but also invigorate conditioned orienting or approach responses via ascending dopaminergic projections to the striatum (Everitt *et al.*, 1999; Han *et al.*, 1997). Our demonstration that the NAcc core and its dopaminergic innervation are critical substrates of appetitive pavlovian approach behaviour (Everitt *et al.*, 1999; Parkinson *et al.*, in press-a), together with the marked impact of a CeN-dorsal striatal dopaminergic disconnection on conditioned orienting (Han *et al.*, 1997), provides a neuroanatomical basis for the integrated functioning of the CeN, the nucleus accumbens and dorsal striatum via the projections from the CeN to midbrain dopamine neurons.

The amygdaloid complex may therefore affect emotional processes by several mechanisms that have evolved through an organism's need to respond adaptively to affective events in its environment. The CeN increasingly is seen as having a quite complex set of functions, which might conveniently be thought of as providing at least two of these mechanisms: (i) to co-ordinate specific autonomic, endocrine and reflexive behavioural responses to cues of emotional significance; this depends upon hypothalamic and brainstem projections. (ii) To energize more general processes, such as the vigour of behavioural responses (activation), enhancing attention and signalling reward, all of which depend upon projections to the isodendritic core. Both (i) and (ii) are, of course, components of the unconditioned response to the US, being consistent with a postulated involvement of the CeN in CS-UR representations. However Holland and co-workers (Holland, 1984; Holland *et al.*, 1999) have argued that motor responses directed towards the US,

rather than the CS, have a separate neural basis and one that appears to be independent of the amygdala. A similar conclusion has been reached concerning aversive eye-blink conditioning, which also survives lesions of either central or basolateral parts of the amygdala (Lavond *et al.*, 1993). A third mechanism depends upon the BLA, which subserves more complex representations of the affective value or specific sensory attribution of conditioned stimuli, thereby enabling goal-directed actions to be influenced via its projections to the prefrontal cortex and ventral striatum, as well as recruiting the diverse processes regulated by the CeN that will also impact on the vigour of such actions.

Implications for addiction and drug-dependence

Associative conditioning clearly comprises several distinct mechanisms, some of which we have shown to depend upon dissociable regions of the amygdala, thereby allowing this structure to influence the complex processes underlying emotional behaviour. While we have been focusing on the involvement of the amygdala in learning involving appetitive and aversive natural stimuli, it is plausible that the mechanisms we have identified may also apply to other reinforcers, such as drugs of abuse. Several theorists have argued strongly that different forms of conditioning contribute to the persistence of compulsive drug use in humans (Childress *et al.*, 1992; Di Chiara, 1998; Everitt, 1996; Everitt *et al.*, 1999; O'Brien *et al.*, 1992a; O'Brien *et al.*, 1992b; Robbins *et al.*, 1999; Robinson *et al.*, 1993; Stewart *et al.*, 1984; Wikler, 1965). Wikler, in his two-factor theory of addiction, argued that the pavlovian association of specific environments with opiate withdrawal provided the basis for the phenomenon of conditioned withdrawal (Wikler, 1965). According to this theory, drug-abstinent (detoxified) individuals, even those who have been abstinent for a relatively long time, experience a conditioned withdrawal syndrome sufficient to induce drug-craving and relapse to heroin self-administration on entering an environment previously paired with opiate withdrawal. There are both clinical and experimental demonstrations of this phenomenon (Childress *et al.*, 1988; Goldberg *et al.*, 1967), which has also been proposed to explain why veterans of the Vietnam war, many of whom were dependent upon heroin while in Vietnam, did not in any numbers relapse to heroin abuse on their return to the USA because of the change in context, i.e. because the heroin withdrawal-associated environmental cues were no longer present to precipitate conditioned withdrawal and thereby relapse to heroin abuse (Robins *et al.*, 1975). Other conditioning theories have emphasized the association of environmental stimuli with the positive reinforcing effects of abused drugs, especially cocaine, but also opiates (Di Chiara, 1998; Robinson *et al.*, 1993; Stewart *et al.*, 1984). While sometimes resulting in conditioned 'highs', in which the ritual of intravenous injection of vehicle alone is sufficient to induce some of the responses usually only seen to the injected drug (O'Brien *et al.*, 1986), specific drug-associated cues are also well known to induce drug-craving in former cocaine-dependent subjects and to be a major contributory factor in the maintenance and reinstatement of a drug-taking habit (Gawin, 1991; O'Brien *et al.*, 1996). In a variant of conditioned positive reinforcement theories of addiction, Robinson and Berridge have argued that the attribution of what they term 'incentive salience' to environmental cues associated with drug effects, imbues them with the power to induce 'drug wanting' (Robinson *et al.*, 1993).

In our own experiments, some of which are reviewed here, we have demonstrated that amphetamine and other stimulant drugs clearly exaggerate the motivational effects of CSs by increasing dopamine transmission in the ventral striatum. Indeed, this may be a major component of the reinforcing effects of such drugs. Since this effect of amphetamine depends upon associative information derived from the amygdala, we see the importance of trying to understand the ways in which associative learning, which might be seen as maladaptive, could lead to the progressive development of the complex syndrome of drug addiction (Robbins *et al.*, 1999). Such maladaptive learning might, then, result in the establishment of compulsive drug use, in which drug-associated cues powerfully induce drug-craving and relapse to drug-seeking behaviour following abstinence (Gawin, 1991; O'Brien *et al.*, 1996). Thus, for example, the simple associative influence exerted by the CeN over ascending arousal systems may provide a key mechanism underlying the increased salience, attractiveness and motivational properties of drug-associated cues. Operating in parallel, by mediating representations of the CS-US (drug) association, the BLA may impact upon processes of conditioned reinforcement whereby drug cues can support and direct instrumental acts having the goal of procuring drugs such as cocaine for self-administration. Relapse to drug addiction precipitated by exposure to drug-associated cues can also be seen as part of the vicious circle of cocaine addiction, which is completed by the effect of the self-administered drug to boost further the dopaminergic processes within the CeN and NAcc that support conditioning to environmental stimuli while simultaneously enhancing their impact on craving and drug-seeking behaviour (Everitt *et al.*, 1999; Phillips *et al.*, 1990).

In order to test this latter hypothesis, we have successfully developed a second-order schedule of intravenous (i.v.) cocaine reinforcement to investigate the neural and pharmacological basis of cue-controlled cocaine-seeking behaviour in rats (Arroyo *et al.*, 1998; Pilla *et al.*, 1999; Weissenborn *et al.*, 1997; Whitelaw *et al.*, 1996). Rats are trained to self-administer cocaine, which is always paired with a discrete light CS. Subsequently, the CS supports responding for a protracted period prior to the i.v. drug infusion, thus providing a measure of drug-seeking behaviour that is not affected by pharmacological effects of the self-administered drug. The importance of contingent presentations of the cocaine-associated cue in maintaining responding has been demonstrated by the finding that responding decreases to about 40% of the control level following the omission of the CS during each of three daily sessions (despite the fact that i.v. cocaine is still self-administered at the end of each of 5 daily fixed-intervals of responding). Responding is promptly re-established at baseline levels following re-introduction of the CS (Arroyo *et al.*, 1998). Presentations of the cocaine cue following extinction of responding are also able to reinstate drug-seeking behaviour, thereby providing a model of cue-induced relapse (Arroyo *et al.*, 1998).

Rats with bilateral, excitotoxic lesions of the BLA could not acquire adequate levels of responding under a second-order schedule of cocaine reinforcement (Whitelaw *et al.*, 1996). Thus, the acquisition of drug-seeking behaviour that depends upon presentation of drug-associated cues requires the integrity of this part of the amygdaloid complex, as does the acquisition of a new response with conditioned reinforcement. However, BLA lesions did not impair the acquisition of i.v. cocaine self-administration *per*

se, indicating that the primary rewarding effects of cocaine do not depend upon the BLA (Whitelaw *et al.*, 1996). Only when responding depended upon presentation of the drug CS was the impact of BLA lesions revealed, consistent with the hypothesis that the BLA is essential for the CS to elicit the affective representation of the primary reinforcer (in this case, cocaine) and consistent with other data reviewed above for appetitive conditioning in general (Burns *et al.*, 1993; Cador *et al.*, 1989; Everitt *et al.*, 1989; Hatfield *et al.*, 1996; Whitelaw *et al.*, 1996). Moreover, cocaine-associated cues are ineffective in reinstating cocaine self-administration in rats with bilateral BLA lesions (Meil *et al.*, 1997), further emphasizing importance of this structure in mediating the effects of the drug-associated CS on drug-taking behaviour. Taken together, these data strongly suggest that the BLA is part of the neural mechanism through which drug-associated CSs can control instrumental behaviour, presumably by interacting directly or indirectly with the ventral striatum, a primary site for the rewarding effects of cocaine and amphetamine (Everitt *et al.*, 1999). Interestingly, rats responding at high rates for heroin under a second-order schedule of reinforcement do not depend critically on contingent presentations of heroin-associated cues and are relatively not affected by CS omission in the way that rats responding for cocaine are (Alderson *et al.*, 1997). Lesions of the BLA, identical to those that prevented the acquisition of cue-controlled cocaine-seeking behaviour, were without effect on the acquisition of this form of heroin-seeking behaviour, further emphasizing the importance of the BLA only for behaviour under the control of CSs associated with the self-administered drug (Alderson, Robbins and Everitt, to be published).

Thus, the involvement of the amygdala in the complex processes underlying addictive behaviour appears to be quite specific. In addition to the demonstrations summarized above showing that the BLA is important for cue-controlled cocaine-seeking behaviour, reinstatement of cocaine self-administration following extinction and for conditioned reinforcement, it has additionally been shown that a conditioned place preference for an environment paired with cocaine also depends upon the BLA (Brown *et al.*, 1993) and that exposure to a cocaine-paired environment induces the expression of c-*fos* in the amygdala, among other limbic cortical structures (Brown *et al.*, 1992). However, other responses are not affected by BLA lesions, for example: (i) conditioned locomotor activity induced by exposing rats to an environment in which they had repeatedly received injections of cocaine; (ii) the unconditioned locomotor response to cocaine (Brown *et al.*, 1993) or amphetamine (Burns *et al.*, 1993; Robledo *et al.*, 1996); (iii) the sensitization of locomotor activity to stimulant drugs, which instead depends upon glutamatergic projections from the prefrontal cortex to the ventral tegmental area (Kalivas *et al.*, 1999; Pierce *et al.*, 1997; Pierce *et al.*, 1998; Wolf, 1998). Thus, BLA lesions only attenuate the acquisition of appetitive behavioural responses that are in large measure under the control of discrete drug-paired stimuli.

Apparently quite different, non-associative influences on cocaine self-administration are seen following dopaminergic manipulations of the amygdala. While 6-hydroxydopamine-induced lesions of the amygdala have only minor effects on cocaine self-administration (McGregor *et al.*, 1994), infusions of dopamine D1 receptor antagonists into the amygdala both increase cocaine self-administration (Caine *et al.*, 1995; Hurd *et al.*, 1997; McGregor *et al.*, 1993) and simultaneously increase dopamine levels in the NAcc

(Hurd *et al.*, 1997). These effects of manipulating dopamine transmission in the amygdala are generally similar to those following identical manipulations of dopamine transmission in the NAcc (Caine *et al.*, 1995; Hurd *et al.*, 1997; McGregor *et al.*, 1993), save that infusions of a D1 receptor antagonist into the NAcc also increased the break point for cocaine self-administration under a progressive ratio schedule, whereas intra-amygdala infusions did not (McGregor *et al.*, 1993). It seems likely that these effects of dopaminergic treatments in the amygdala are mediated indirectly by the CeN (which receives the richest dopaminergic innervation among amygdala sub-regions) via its midbrain projections that can regulate the activity of mesolimbic dopamine neurons and hence dopamine levels in the NAcc. Such interactions may be considered within the conceptual framework of the extended amygdala, which has been strongly implicated in mediating the reinforcing effects of drugs of abuse (Koob, 1999), or may reflect the influence of the CeN upon ascending dopaminergic transmission (Everitt *et al.*, 1999).

In studies parallel to those on associative processes influencing cocaine self-administration, we have also shown that the BLA is important in mediating the depressant effects on appetitive behaviour of stimuli associated with heroin withdrawal (Everitt *et al.*, 1993). In these experiments, rats were trained to lever press at high rates for food on a fixed interval schedule. They were then made opiate-dependent by implanting them subcutaneously with morphine pellets and their baseline levels of responding for food was re-established. Subsequently, acute withdrawal was precipitated on four occasions by injection of low doses of the opiate antagonist, naloxone, in the presence of a distinctive visual/auditory CS. Following these four conditioning trials, the rats were again allowed to respond for food and the effects on responding of presentations of the withdrawal-associated CS were measured. The marked suppression of responding induced by the withdrawal-associated CS in control rats was not observed in rats that had received excitotoxic lesions of the BLA prior to conditioning, even though these lesions did not alter the unconditioned withdrawal precipitated by naloxone in dependent rats. These results are again consistent with the hypothesis that the BLA is essential for retrieval by the CS of the affective representation of the primary reinforcer – in this case the aversive state of withdrawal. They also emphasize that the associative mechanisms subserved by the amygdala are independent of the positive or negative affective valence of the reinforcer.

Functional imaging studies of human cocaine and heroin abusers have demonstrated that exposure to cocaine cues, including the paraphernalia associated with drug self-administration and videos of drug use, results in significant activation of medial temporal lobe structures, which is at least partly attributable to the amygdala (Childress *et al.*, 1999; Grant *et al.*, 1996). It is perhaps important to note that these same studies reveal the activation of a cortical network on exposure to drug cues that includes the anterior cingulate, orbitofrontal and dorsolateral prefrontal cortices, but not the striatum. The latter is, however, strongly affected by self-administered cocaine or by other dopaminergic drugs (Breiter *et al.*, 1996; Volkow *et al.*, 1996; Volkow *et al.*, 1997a; Volkow *et al.*, 1997b), frequently in a way that is correlated with indices of craving. As in animal experimental studies, these data suggest that the neural mechanisms mediating the primary reinforcing effects of abused drugs can be dissociated from those mediating conditioned

reinforcement and the impact of drug-conditioned cues on drug-craving. This realisation suggests that pharmacological or other interference with the mechanisms by which drug cues induce craving and drug-seeking behaviour might present a therapeutic target for drugs having utility in the treatment of this aspect of cocaine addiction. Indeed, we have shown that a dopamine D3 receptor partial agonist, BP 897, which has no primary rewarding effects, selectively reduces cocaine cue-dependent responding under a second-order schedule of cocaine self-administration in rats (Pilla *et al.*, 1999). It remains to be seen whether such a compound is able to diminish the propensity of drug-associated cues to induce relapse to drug-taking in otherwise abstinent cocaine or other drug abusers.

Conclusion

The results presented here, taken together with a growing literature, support the notion of functionally dissociable amygdala sub-systems that are involved in associative processes underlying both appetitive and aversive emotional behaviour. We have suggested that apparent discrepancies in the larger database can be resolved around the hypothesis that the CeN and its associated circuitry underlies conditioned motivational influences on behaviour, whereas the BLA may provide a more complex representational role in emotionally charged decisions and voluntary behaviour. This analysis of amygdala function has wide-ranging implications for understanding the neural basis of emotional behaviour and its disorders, highlighted here by considering the importance of associative processes on the development and persistence of addiction to drugs.

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References

Alderson, H. L., Robbins, T. W., & Everitt, B. J. (1997). Establishment of intravenous self-administration of heroin in the rat under a second-order schedule of reinforcement. Society for Neuroscience Abstracts, **23,** 938.7.

Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid and corticopetal components of the substantia innominata. Neuroscience, **27,** 1-39.

Amaral, D. G., Price, J. L., Pitkänen, A., & Carmichael, S. T. (1992). Anatomical organization of the primate amygdaloid complex. *In* J. P. Aggleton (Ed.), The amygdala: neurobiological aspects of emotion, memory and mental dysfunction pp. 1-66. Wiley-Liss. New York.

Applegate, C. D., Frysinger, R. C., Kapp, B. S., & Gallagher, M. (1982). Multiple unit activity recorded from the amygdala central nucleus during pavlovian heart rate conditioning in the rabbit. Brain Research, **238,** 457-462.

Arroyo, M., Markou, A., Robbins, T. W., & Everitt, B. J. (1998). Acquisition, maintenance and reinstatement of intravenous cocaine self- administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology (Berl), **140,** 331-44.

Balleine, B. (1994). Asymmetrical interactions between thirst and hunger in Pavlovian-instrumental transfer. Quarterly Journal of Experimental Psychology, **47B,** 211-231.

Bolles, R. C., & Fanselow, M. S. (1980). A perceptual-defensive-recuperative model of fear and pain. Behavioral Brain Sciences, **3,** 291-323.

Breiter, H., Gollub, R., Weisskoff, R., Kennedy, D., Kanto, H., Gastfriend, D., Berke, J., Riorden, J., Mathew, T., Makris, N., Guimaraes, A., Rosen, B., & Hyman, S. (1996). Activation of human brain reward circuitry by cocaine observed using fMRI. Society for Neuroscience Abstracts, **22,** 758.6.

Brown, E. E., & Fibiger, H. C. (1993). Differential effects of excitotoxic lesions of the amygdala on cocaine-induced conditioned locomotion and conditioned place preference. Psychopharmacology, **113,** 123-130.

Brown, E. E., Robertson, G. S., & Fibiger, H. C. (1992). Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. Journal of Neuroscience, **12,** 4112-4121.

Burns, L. H., Everitt, B. J., & Robbins, T. W. (1999). Effects of excitotoxic lesions of the basolateral amygdala on conditional discrimination learning with primary and conditioned reinforcement. Behavioural Brain Research, **100,** 123-133.

Burns, L. H., Robbins, T. W., & Everitt, B. J. (1993). Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. Behavioural Brain Research, **55,** 167-183.

Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1997). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: Implications for the neurobiology of emotion. Behavioral Neuroscience, **111,** 908-919.

Cador, M., Robbins, T. W., & Everitt, B. J. (1989). Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience., **30,** 77-86.

Cahill, L., Weinberger, N. M., Roozendaal, B., & McGaugh, J. L. (1999). Is the amygdala a locus of "conditioned fear"? Some questions and caveats. Neuron, **23,** 227-228.

Caine, S. B., Heinrichs, S. C., Coffin, V. L., & Koob, G. F. (1995). Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. Brain Research, **692,** 47-56.

Childress, A. R., Ehrman, R., Roohsenow, D. J., Robbins, S. J., & O'Brien, C. P. (1992). Classically conditioned factors in drug dependence. *In* W. Lowinson, P. Luiz, R. B. Millman, & J. G. Langard (Eds.), Substance abuse: a comprehensive text book pp. 56-69. Williams and Wilkins. Baltimore.

Childress, A. R., McElgin, W., Mozley, P. D., & Obrien, C. P. (1999). Limbic activation during cue-induced craving for cocaine and for natural rewards. Biological Psychiatry, **45,** 170.

Childress, A. R., McLellan, A. T., Ehrman, R., & O'Brien, C. P. (1988). Classically conditioned responses in opioid and cocaine dependence: a role in relapse? NIDA Research Monographs, **84,** 25-43.

Clugnet, M.-C., & LeDoux, J. E. (1990). Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. Journal of Neuroscience, **10,** 2818-2824.

Damasio, A. R., & Damasio, H. (1993). Cortical systems underlying knowledge retrieval: evidence from human lesion studies. *In* T. A. Poggio, & D. A. Glaser (Eds.), Exploring Brain Functions: Models in Neuroscience pp. 233-248. John Wiley & Sons Ltd. Chichester.

Davis, M. (1992a). The role of the amygdala in conditioned fear. *In* J. P. Aggleton (Ed.), The Amygdala pp. 255-306. Wiley-Liss Inc. New York.

Davis, M. (1992b). The role of the amygdala in fear and anxiety. Annual Review of Neuroscience, **15,** 353-75.

Davis, M. (1997). Neurobiology of fear responses: the role of the amygdala. Journal of Neuropsychiatry and Clinical Neuroscience, **9,** 382-402.

De Olmos, J. S., & Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. Annals of the New York Academy of Sciences, **877,** 1-32.

Di Chiara, G. (1998). A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. Journal of Psychopharmacology, **12,** 54-67.

Dickinson, A. (1994). Instrumental conditioning. *In* N. J. Mackintosh (Ed.), Animal Learning and Cognition pp. 45-78. Academic Press. London.

Dickinson, A., & Dearing, M. F. (1979). Appetitive-aversive interactions and inhibitory processes. *In* A. Dickinson, & R. A. Boakes (Eds.), Mechanisms of learning and motivation pp. 203-31. Erlbaum. Hillsdale, NJ.

Everitt, B. J. (1996). Drug cues and craving, actions and habits: the search for a neuropsychological basis of addiction. Medical Research Council News, **Summer,** 11-15.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, **30,** 63-75.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, **42,** 1-18.

Everitt, B. J., Parkinson, J. A., Olmstead, M. C., Arroyo, M., Robledo, P., & Robbins, T. W. (1999). Associative processes in addiction and reward: The role of amygdala-ventral striatal subsystems. Annals New York Academy of Sciences, **877,** 412-438.

Everitt, B. J., & Robbins, T. W. (1992). Amygdala-ventral striatal interactions and reward-related processes. *In* J. P. Aggleton (Ed.), The Amygdala pp. 401-430. John Wiley & Sons Inc. New York.

Everitt, B. J., Schulteis, G., & Koob, G. F. (1993). Attenuation of conditioned opiate withdrawal following excitotoxic lesions of the basolateral amygdala. Society for Neuroscience, **19,** 511.5-0.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. Neuroscience and Biobehavioral Reviews, **23,** 743-760.

Freeman, J. H., Cuppernell, C., Flannery, K., & Gabriel, M. (1996). Limbic thalamic, cingulate cortical and hippocampal neuronal correlates of discriminative approach learning in rabbits. Behavioural Brain Research, **80,** 123-136.

Fuster, J. M. (1995). Memory in the Cerebral Cortex: an empirical approach to neural networks in the human and nonhuman primate. Cambridge, Massachusetts: MIT Press.

Gabriel, M. (1990). Functions of anterior and posterior cingulate cortex during avoidance-learning in rabbits. Progress in Brain Research, **85,** 467-483.

Gabriel, M., Vogt, B. A., Kubota, Y., Poremba, A., & Kang, E. (1991). Training-Stage Related Neuronal Plasticity in Limbic Thalamus and Cingulate Cortex During Learning - a Possible Key to Mnemonic Retrieval. Behavioural Brain Research, **46,** 175-185.

Gaffan, D. (1992). Amygdala and the memory of reward. *In* J. Aggleton, P. (Ed.), The Amygdala pp. 471-484. Wiley &Sons. Chichester.

Gallagher, M., Graham, P. W., & Holland, P. C. (1990). The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behaviour. Journal of Neuroscience, **10,** 1906-1911.

Gallagher, M., & Holland, P. C. (1992). Understanding the functions of the central nucleus: Is simple conditioning enough? *In* J. P. Aggleton (Ed.), The Amygdala pp. 307-322. Wiley and Sons. Chicester.

Gallagher, M., & Holland, P. C. (1994). The amygdala complex: multiple roles in associative learning and attention. Proceedings of the National Academy of Sciences, USA, **91,** 11771-11776.

Gawin, F. H. (1991). Cocaine addiction: psychology and neurophysiology. Science, **251,** 1580-1586.

Gewirtz, J. C., & Davis, M. (1998). Application of Pavlovian higher-order conditioning to the analysis of the neural substrates of fear conditioning. Neuropharmacology, **37,** 453-459.

Goldberg, S. R., & Schuster, C. R. (1967). Conditioned suppression by a stimulus associated with nalorphine in post morphine-dependent monkeys. Journal of the Experimental Analysis of Behavior, **10,** 235-242.

Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Xiang, L., Contoreggi, C., Phillips, R. L., Kimes, A. S., & Margolin, A. (1996). Activation of memory circuits during cue-elicited cocaine craving. Proceedings of the National Academy of Sciences, USA, **93,** 12040-12045.

Groenewegen, H. J., Wright, C. I., & Beijer, A. V. J. (1996). The nucleus accumbens: Gateway for limbic structures to reach the motor system? Progress in Brain Research, **107,** 485-511.

Han, J.-S., Holland, P. C., & Gallagher, M. (in press). Disconnection of amygdala central nucleus and substantia innominata/nucleus basalis disrupts increments in conditioned stimulus processing. Behavioral Neuroscience.

Han, J. S., McMahan, R. W., Holland, P., & Gallagher, M. (1997). The role of an amygdalo-nigrostriatal pathway in associative learning. Journal of Neuroscience, **17,** 3913-3919.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. Journal of Neuroscience, **16,** 5256-5265.

Hearst, E., & Jenkins, H. M. (1974). Sign Tracking: The Stimulus-Reinforcer Relation and Directed Action. Monograph of the Psychonomic Society.

Henke, P. G., Allen, J. D., & Davison, C. (1972). Effects of lesions in the amygdala on behavioral contrast. Physiology and Behavior, **8,** 173-176.

Hiroi, N., & White, N. M. (1991). The Lateral Nucleus of the Amygdala Mediates Expression of the Amphetamine-Produced Conditioned Place Preference. Journal of Neuroscience, **11,** 2107-2116.

Hitchcott, P. K., Bonardi, C. M. T., & Phillips, G. D. (1997a). Enhanced stimulus-reward learning by intra-amygdala administration of a D-3 dopamine receptor agonist. Psychopharmacology, **133,** 240-248.

Hitchcott, P. K., Harmer, C. J., & Phillips, G. D. (1997b). Enhanced acquisition of discriminative approach following intra- amygdala d-amphetamine. Psychopharmacology, **132,** 237-246.

Hitchcott, P. K., & Phillips, G. D. (1998). Double dissociation of the behavioural effects of R(+) 7-OH-DPAT infusions in the central and basolateral amygdala nuclei upon Pavlovian and instrumental conditioned appetitive behaviours. Psychopharmacology, **140,** 458-69.

Holland, P. C. (1984). The origins of Pavlovian conditioned behavior. *In* G. Bower (Ed.), The Psychology of Learning and Motivation pp. 129-173. Prentice-Hall. .

Holland, P. C., & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. Trends in Cognitive Sciences, **3,** 65-73.

Hurd, Y. L., McGregor, A., & Ponten, M. (1997). In vivo amygdala dopamine levels modulate cocaine self-administration behaviour in the rat: D1 dopamine receptor involvement. European Journal of Neuroscience, **9,** 2541-2548.

Inglis, W. L., Olmstead, M. C., & Robbins, T. W. (in press). Pedunculopontine tegmental nucleus lesions impair stimulus-reward learning in autoshaping and conditioned reinforcement paradigms. Behavioral Neuroscience.

Kalivas, P. W., & Nakamura, M. (1999). Neural systems for behavioral activation and reward. Current Opinion in Neurobiology, **9,** 223-227.

Kelley, A. E., & Domesick, V. B. (1982a). The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. Neuroscience, **7,** 2321-2335.

Kelley, A. E., Domesick, V. B., & Nauta, W. J. H. (1982b). The amygdalostriatal projection in the rat - an anatomical study by anterogrde and retrograde tracing methods. Neuroscience, **7,** 615-630.

Killcross, A. S. (1998). Dissociable effects of excitotoxic lesions of amygdala sub-nuclei on appetitive conditoning. Journal of Psychopharmacology, **12,** A4.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997a). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, **388,** 377-380.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997b). Is it time to invoke multiple fear systems in the amygdala?: a reply to Nader and LeDoux. Trends in Cognitive Sciences, **1,** 244-247.

Kluver, H., & Bucy, P. C. (1939). Preliminary analysis of functions of the temporal lobes in monkeys. Archives of Neurology and Psychiatry, **8,** 2153-2163.

Konorski, J. (1948). Conditioned reflexes and neuron organization. Cambridge, UK: Cambridge University Press.

Konorski, J. (1967). Integrative activity of the brain. Chicago, Illinois: University of Chicago Press.

Koob, G. F. (1999). The role of the striatopallidal and extended amygdala systems in drug addiction. Annals of the New York Academy of Sciences, **877,** 445-460.

Krettek, J. E., & Price, J. L. (1978). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. Journal of Comparative Neurology, **178,** 225-253.

Lagowska, J., & Fonberg, E. (1975). Salivary reactions in dogs with dorsomedial amygdalar lesions. Acta Neurobiologica Experientia, **35,** 17-26.

Lavond, D. G., Kim, J. J., & Thompson, R. F. (1993). Mammalian brain substrates of aversive classical conditioning. Annual Reviews of Psychology, **44,** 317-342.

LeDoux, J. (1996). The emotional brain. New York: Simon & Schuster.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. Journal of Neuroscience, **10,** 1062-1069.

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reid, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. The Journal of Neuroscience, **8,** 2517-2529.

Louilot, A., Simon, H., Taghzouti, K., & Le Moal, M. (1985). Modulation of dopaminergic activity in the nucleus accumbens following facilitation or blockade of the dopaminergic transmission in the amygdala: A study by in vivo differential pulse voltammetry. Brain Research, **346,** 141-145.

Lovibond, P. F. (1983). Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. Journal of Experimental Psychology: Animal Behavior Processes, **9,** 225-247.

Mackintosh, N. J. (1974). The Psychology of Animal Learning. London: Academic Press.

Mackintosh, N. J. (1983). Conditioning and Associative Learning. Oxford: Oxford University Press.

Maren, S. (1996). Synaptic transmission and plasticity in the amygdala - An emerging physiology of fear conditioning circuits. Molecular Neurobiology, **13,** 1-22.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behavioural Brain Research, **88,** 261-274.

Maren, S., & Fanselow, M. S. (1996). The amygdala and fear conditioning: has the nut been cracked? Neuron, **16,** 237-240.

McAlonan, G. M., Robbins, T. W., & Everitt, B. J. (1993). Effects of medial dorsal thalamic and ventral pallidal lesions on the acquisition of a conditioned place preference: Further evidence for the involvement of the ventral striatopallidal system in reward- related processes. Neuroscience, **52,** 605-620.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. Progress in Neurobiology, **55,** 257-332.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: hippocampus, amygdala and dorsal striatum. Behavioral Neuroscience, **107,** 3-22.

McGregor, A., Baker, G., & Roberts, D. C. S. (1994). Effect of 6-hydroxydopamine lesions of the amygdala on intravenous cocaine self-administration under a progressive ratio schedule of reinforcement. Brain Research, **646,** 273-278.

McGregor, A., & Roberts, D. C. S. (1993). Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Research, **624,** 245-252.

McKernan, M., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature, **390,** 604-607.

Meil, W. M., & See, R. E. (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behavioural Brain Research, **1997,** 139-148.

Mogenson, G., Jones, D. L., & Yim, C. Y. (1984). From motivation to action: functional interface between the limbic system and the motor system. Progress in Neurobiology, **14,** 69-97.

Nader, K., & LeDoux, J. (1997). Is it time to invoke multiple fear systems in the amygdala? Trends in Cognitive Sciences, **1,** 241-243.

O'Brien, C. P., Childress, A. R., McLellan, A. T., & Ehrman, R. (1992a). Classical conditioning in drug-dependent humans. Annals of the New York Academy of Sciences, **654,** 400-415.

O'Brien, C. P., Childress, A. R., McLellan, A. T., & Ehrman, R. (1992b). A learning model of addiction. Research Publications of the Association for Research on Nervous and Mental Diseases., **70,** 157-177.

O'Brien, C. P., Ehrman, R. N., & Ternes, J. W. (1986). Classical conditioning in human opioid dependence. *In* S. R. Goldberg, & I. P. Stolerman (Eds.), Behavioral Analysis of Drug Dependence pp. 329-356. Academic Press Inc. London.

O'Brien, C. P., & McLellan, A. T. (1996). Myths about the treatment of addiction. Lancet, **347,** 237-240.

Parkinson, J. A., Olmstead, M. C., Burns, L. H., Robbins, T. W., & Everitt, B. J. (1999). Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. Journal of Neuroscience, **19,** 2401-2411.

Parkinson, J. A., Willoughby, P. J., Robbins, T. W., & Everitt, B. J. (in press-a). Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortico-ventral striatopallidal systems. Behavioral Neuroscience.

Parkinson, J. P., Robbins, T. W., & Everitt, B. J. (in press-b). Selective excitotoxic lesions of the nucleus accumbens core and shell differentially affect aversive Pavlovian conditioning to discrete and contextual cues. Psychobiology.

Pascoe, J. P., & Kapp, B. S. (1985a). Electrophysiological characteristics of amygdaloid central nucleus neurons during pavlovian fear conditioning in the rabbit. Behavioral Brain Research, **16,** 117-133.

Pascoe, J. P., & Kapp, B. S. (1985b). Electrophysiological characteristics of amygdaloid central nucleus neurons in the awake rabbit. Brain Research Bulletin, **14,** 331-338.

Phillips, A. G., & Fibiger, H. C. (1990). Role of reward and enhancement of conditioned reward in persistence of responding for cocaine. Behavioural Pharmacology, **1,** 269-282.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Research Reviews, **25,** 192-216.

Pierce, R. C., Reeder, D. C., Hicks, J., Morgan, Z. R., & Kalivas, P. W. (1998). Ibotenic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine. Neuroscience, **82,** 1103-1114.

Pilla, M., Perachon, S., Sautel, F., Garrido, F., Mann, A., Wermuth, C. G., Schwartz, J.-C., Everitt, B. J., & Sokoloff, P. (1999). Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature, **400,** 371-375.

Price, J. L., Russchen, F. T., & Amaral, D. (1987). The Limbic Region. II: The Amygdaloid Complex. *In* A. Björklund, T. Hökfelt, & L. W. Swanson (Eds.), Handbook of Chemical Neuroanatomy, Volume 5: Integrated Systems of the C.N.S., Part 1 pp. 279-388. Elsevier. Amsterdam.

Robbins, T. W. (1978). The acquisition of responding with conditioned reinforcement: Effects of pipradrol, methylphenidate, d-amphetamine and nomifensine. Psychopharmacology, **58,** 79-87.

Robbins, T. W., Cador, M., Taylor, J. R., & Everitt, B. J. (1989). Limbic-striatal interactions in reward-related processes. Neuroscience and Biobehavioral Reviews, **13,** 155-162.

Robbins, T. W., & Everitt, B. J. (1999). Drug addiction: bad habits add up. Nature, **398,** 567-570.

Robins, L. N., Helzer, J. E., & Davis, D. H. (1975). Narcotic use in southeast Asia and afterwards. Archives of General Psychiatry, **32,** 955-961.

Robinson, T. E., & Berridge, K. C. (1993). The Neural Basis of Drug Craving - an Incentive-Sensitization Theory of Addiction. Brain Research Reviews, **18,** 247-291.

Robledo, P., Robbins, T. W., & Everitt, B. J. (1996). Effects of excitotoxic lesions of the central amygdaloid nucleus on the potentiation of reward-related stimuli by intra-accumbens amphetamine. Behavioral Neuroscience, **110,** 981-990.

Rolls, E. T. (1999). The Brain and Emotion. Oxford: Oxford University Press.

Roozendaal, B., Oldenburger, W. P., Strubbe, J. H., Koolhaas, J. M., & Bohus, B. (1990). The central amygdala is involved in the conditioned but not in the meal- induced cephalic insulin response in the rat. Neuroscience Letters, **116,** 210-5.

Sarter, M., & Markowitsch, H. (1985). Involvement of the amygdala in learning and memory: a critical review with emphasis on anatomical relations. Behavioral Neuroscience, **99,** 342-380.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1998). Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nature Neuroscience, **1,** 155-9.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. Journal of Neuroscience, **19,** 1876-1884.

Schwartzbaum, J. S. (1965). Discrimination behavior after amygdalectomy in monkeys: visual and somesthetic learning and perceptual capacity. Journal of Comparative and Physiological Psychology, **60,** 314-319.

Selden, N. R. W., Everitt, B. J., Jarrard, L. E., & Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. Neuroscience, **42,** 335-350.

Simon, H., Taghzouti, K., Gozlan, H., Studler, J. M., Louilot, A., Herve, D., Glowinski, J., Tassin, J. P., & Le Moal, M. (1988). Lesion of dopaminergic terminals in the amygdala produces enhanced locomotor response to D-amphetamine and opposite changes in dopaminergic activity in prefrontal cortex and nucleus accumbens. Brain Research, **447,** 335-340.

Smith, J. W., & Dickinson, A. (1998). The dopamine antagonist, pimozide, abolishes Pavlovian-instrumental transfer. Journal of Psychopharmacology, **12,** A6.

Stewart, J., de Wit, H., & Eikelboom, R. (1984). The role of unconditioned and conditioned drug effects in the self administration of opiates and stimulants. Psychological Reviews, **91,** 251-268.

Swanson, L. W., & Mogenson, G. J. (1981). Neural mechanisms for the functional coupling of autonomic, endocrine and somatomotor responses in adaptive behavior. Brain Research Reviews, **3,** 1-34.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? Trends in Neurosciences, **21,** 323-31.

Taylor, J. R., & Robbins, T. W. (1984). Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology, **84,** 405-412.

Taylor, J. R., & Robbins, T. W. (1986). 6-hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacology, **90,** 390-397.

Tomie, A. (1996). Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neuroscience and Biobehavioural Reviews, **20,** 505-535.

Tomie, A., Brooks, W., & Zito, B. (1989). Sign-tracking: the search for reward. *In* S. B. Klein, & R. R. Mowrer (Eds.), Contemporary Learning Theories - Pavlovian conditioning and the status of traditional learning theory pp. 191-226. Lawrence Erlbaum Associates. Hillsdale, NJ.

Trapold, M. A. (1970). Are expectancies based upon different positive reinforcing events discriminably different? Learning and Motivation, **1,** 129-140.

Vazdarjanova, A., & McGaugh, J. L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proceedings of the New York Academy of Sciences, **95,** 15003-15007.

Vogt, B. A. (1985). Cingulate cortex. *In* E. G. Jones, & A. Peters (Eds.), Cerebral Cortex pp. 89-149. Plenum Press. New York.

Volkow, N. D., Ding, Y.-S., Fowler, J. S., & Wang, G.-J. (1996). Cocaine addiction: hypothesis derived from imaging studies with PET. Journal of Addictive Diseases, **15,** 55-71.

Volkow, N. D., Wang, G. J., Fischman, M. W., Foltin, R. W., Fowler, J. S., Abumrad, N. N., Vitkun, S., Logan, J., Gatley, S. J., Pappas, N., Hitzemann, R., & Shea, C. E. (1997a). Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature, **386,** 827-830.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Hitzemann, R., Chen, A. D., Dewey, S. L., & Pappas, N. (1997b). Decreased striatal dopaminergic responsiveness in detoxified cocaine- dependent subjects. Nature, **386,** 830-833.

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. Journal of Neuroscience, **17,** 9375-9383.

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. Journal of Comparative and Physiological Psychology, **49,** 381-391.

Weissenborn, R., Robbins, T. W., & Everitt, B. J. (1997). Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. Psychopharmacology, **134,** 242-257.

Whitelaw, R. B., Markou, A., Robbins, T. W., & Everitt, B. J. (1996). Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. Psychopharmacology, **127,** 213-224.

Wikler, A. (1965). Conditioning factors in opiate addiction and relapse. *In* D. I. Willner, & G. G. Kassenbaum (Eds.), Narcotics pp. 7-21. McGraw-Hill. New York.

Williams, D. R., & Williams, H. (1969). Auto-maintenance in the pigeon: sustained pecking despite contingent non-reinforcement. Journal of the Experimental Analysis of Behavior, **12,** 511-520.

Wolf, M. E. (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Progress in Neurobiology, **54,** 679-720.

Wolterink, G., Phillips, G., Cador, M., DonselaarWolterink, I., Robbins, T. W., & Everitt, B. J. (1993). Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. Psychopharmacology, **110,** 355-364.

Zahm, D. S., Jensen, S. L., Williams, E. S., & Martin, I., J.R. (1999). Direct comparison of projections from the central amygdaloid region and nucleus accumbens shell. European Journal of Neuroscience, **11,** 1119-1126.

Table 1

Summary of some of the effects of BLA and CeN lesions on different forms of association and the behavioural procedures used to demonstrate them.

Table 1

Association Type	***Lesion***	***Behaviour sensitive to lesion***
CS-UR	**CeN**	Acquisition of Autoshaping
		Acquisition of Conditioned Orienting
		Conditioned Salivation
		Pavlovian-to-Instrumental transfer
CS-US	**BLA**	Second Order Pavlovian Responding
		Second Order Instrumental Responding
		Conditioned Reinforcement
		Conditioned Place or Cue Preference / Avoidance
		Conditioned Freezing *
		Conditioned Startle *

* Note that these behaviours are also sensitive to lesions of the CeA.

Figure 1

The effects of lesions of the CeN and BLA on a pavlovian approach behavior measured in an autoshaping task (left panels) and the acquisition of a new response with conditioned reinforcement (right panel). Panels A and B show the acquisition of autoshaping in BLA lesioned and control subjects (A) and CeN lesioned and control subjects (B). It can be seen that, over 10 blocks of 10 trials, control subjects come selectively to approach the CS+ (which is paired with food reward) and to withhold approaches to the CS-. This is also expressed as the difference between CS+ vs CS- approaches (Difference Score). Rats with BLA lesions are unimpaired (A), whereas rats with CeN lesions do not acquire discriminated approach to the CS+ over the 10 blocks of trials (B). The right panel shows the effects of lesions of the CeN and BLA on the acquisition of a new response with conditioned reinforcement. A CS associated with sucrose in a pavlovian stage is then presented contingent upon responding on 1 of 2 levers, the CRf lever (shaded bars); responding on the second lever (open bars) has no programmed consequence. No sucrose is available at this stage and the conditioned reinforcing properties of the CS are assessed by its ability to support the learning of this new instrumental response. Control subjects respond selectively upon the CRf lever, as do rats with lesions of the CeN. However, rats with BLA lesions are impaired in acquisition, i.e. do not respond selectively on the lever that produces the conditioned reinforcer. The data have been taken from (Burns *et al.,* 1993; Robledo *et al.*, 1996). Thus, in this figure, the double dissociation of the effects of CeN and BLA lesions on discriminated approach and conditioned reinforcement is shown.

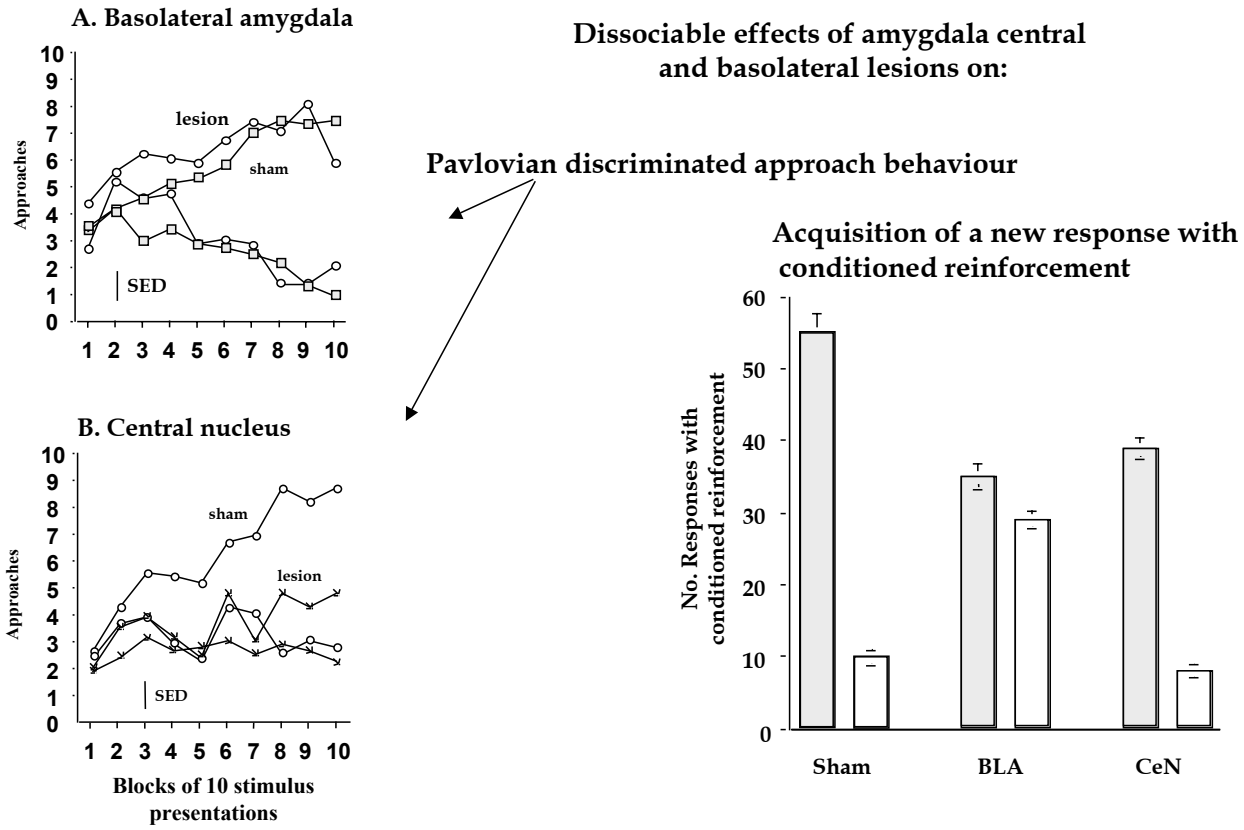

Figure 2

A schematic diagram of the neural network underlying aspects of pavlovian conditioning approach based upon our own data discussed in the text and also data from Gallagher, Holland and co-workers (Gallagher *et al.*, 1994; Holland *et al.*, 1999). The anterior cingulate cortex (ACg), NAcc core, CeN and also the dopaminergic innervation of the NAcc are all necessary, but not sufficient, to support the development of pavlovian approach behavior. We hypothesize that the anterior cingulate cortex and NAcc core are part of a cortico-striatal loop that subserves conditioning per se, i.e. subserves informational processes that give direction to approach behavior. We also hypothesize that there is a relationship between the CeN and ventral tegmental area (VTA) dopamine neurons innervating the NAcc which is also engaged by appetitive CSs to activate, or "energize" pavlovian approach response tendencies (see text for details). In separate, but related experiments, Gallagher, Holland and colleagues have shown that the CeN is critical for conditioned orienting responses, which depends upon interactions of the CeN with the nigrostriatal dopamine system, and for attentional processes which depend upon interactions with the cholinergic nucleus basalis magnocellularis (NBM). (Figure reproduced from (Everitt *et al.*, 1999).

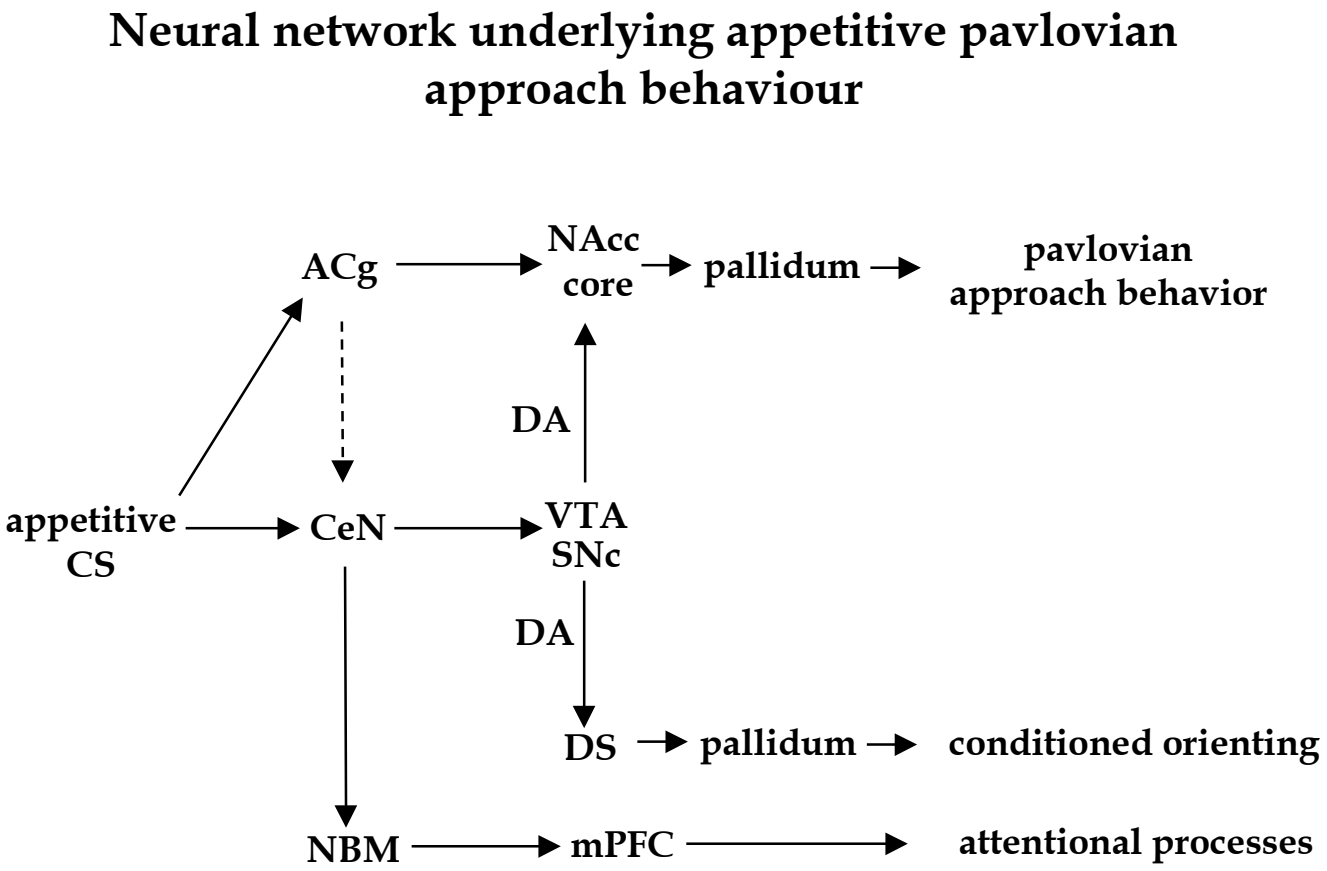

Figure 3.
A schematic illustrating some of the outputs of the CeN. Projections to autonomic, neuroendocrine and primitive motor domains of the hypothalamus and brainstem are well known (see text). The CeN also has rich projections to the chemically defined systems of the isodendritic core, namely the nucleus basalis in the basal forebrain (cholinergic), ventral tegmental area/substantia nigra (dopaminergic), midbrain raphé nuclei (serotoninergic) and pontine locus ceruleus (noradrenergic). Through these routes, the CeN is able to bring widespread areas of the forebrain under its control, thereby influencing component arousal processes including motor activation and attention.

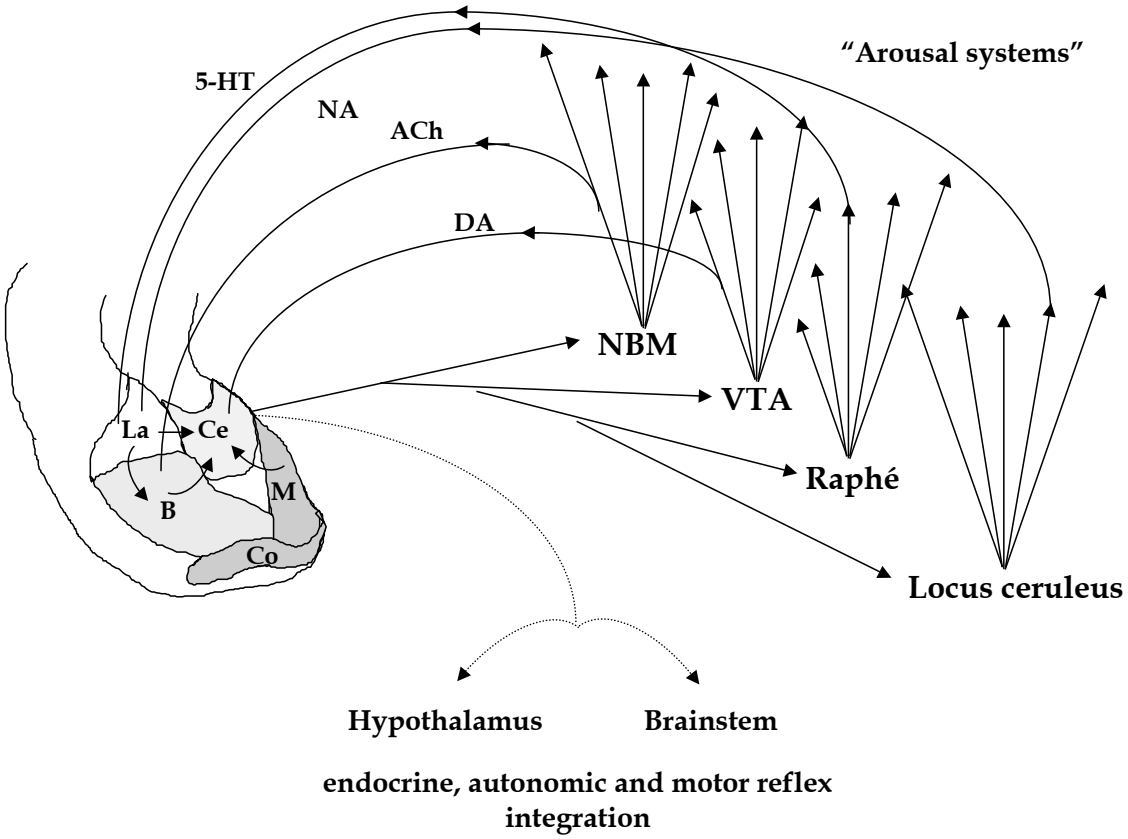

Figure 4.
Pavlovian to instrumental transfer in animals with lesions of the BLA (A) and CeN (B). Subjects were trained to associate a CS with food reinforcement and were then trained to lever-press for the same reinforcer. In normal subjects, presentation of the food-associated CS enhances lever-pressing in a test session - the pavlovian to instrumental transfer effect. Control subjects in both experiments showed this enhancement of lever pressing during the presentation of the pavlovian CS at test, illustrated here as the ratio of lever responses during the CS presentation compared to the total responses during the CS and inter-stimulus interval. Rats with lesions of the BLA also showed normal pavlovian to instrumental transfer. However rats with lesions of the CeN failed to enhance their lever responding during the CS, showing a ratio of responding equivalent to that expected by chance performance (represented by the dashed line on the graph). This suggests that the CeN, but not the BLA, is part of a neural system required to mediate the excitatory effects of pavlovian stimuli on instrumental behaviour.

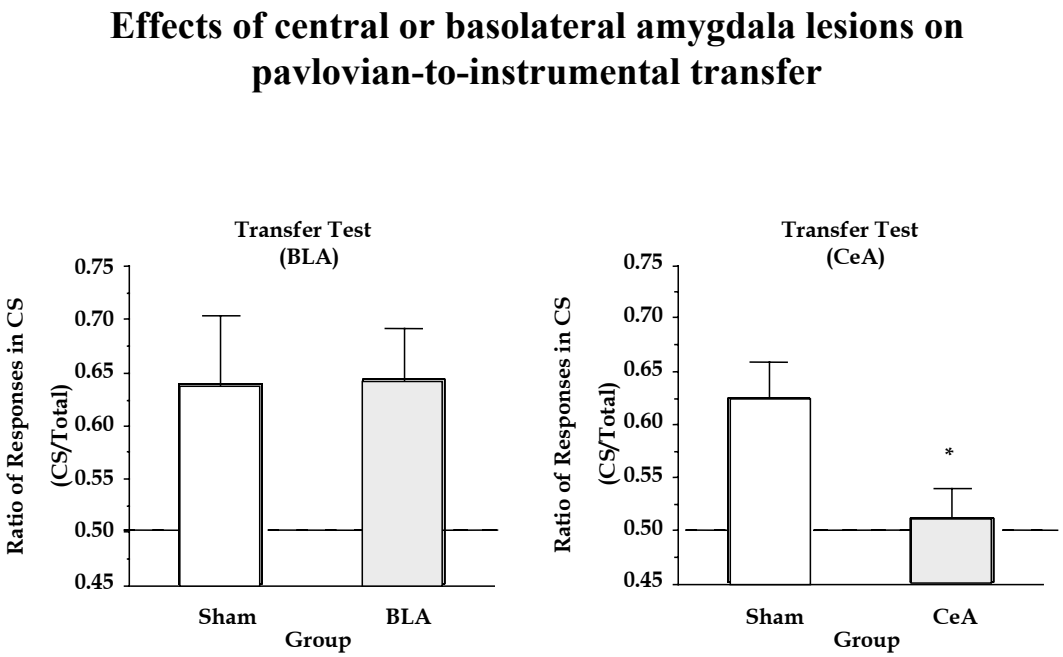

Figure 5
A schematic diagram of the neural network underlying conditioned reinforcement and its potentiation by intra-accumbens d-amphetamine based upon the data presented from (Burns *et al.*, 1993; Cador *et al.*, 1989; Everitt *et al.*, 1999; Parkinson *et al.*, 1999; Robledo *et al.*, 1996; Taylor *et al.*, 1984; Taylor *et al.*, 1986). The conditioned reinforcement process, whereby a pavlovian CS+ supports the acquisition of a new response as a conditioned reinforcer (see Figure 1), depends upon the integrity of the BLA and NAcc core (CORE); there are rich projections from the BLA to this area of the NAcc. Lesions of either BLA or NAcc core do not directly affect the potentiative effects of d-amphetamine, other than by reducing the conditioned reinforcement effect itself. By contrast, the NAcc shell (SHELL) and CeN appear to be critical substrates for the potentiative effect of d-amphetamine on CRf, but not the control over instrumental behaviour by the CS, acting therefore as a conditioned reinforcer. We hypothesize that the commonality in effects of NAcc shell and CeN lesions, supportive of the "extended amygdala" concept, depends upon regulatory influences of the CeN on the dopaminergic innervation of the NAcc shell via projections to the VTA dopamine cell bodies. The ventral subiculum is also critical for the effects of intra-NAcc d-amphetamine on conditioned reinforcement. We hypothesize that this influence of the ventral subiculum depends upon known projections to the caudomedial NAcc shell. Whilst the BLA and CeN appear to be especially concerned with discrete CS processing, we further hypothesize, based on neuropsychological data, that the ventral subiculum provides contextual information upon which the CRf potentiation effect is based. Clearly, then, there is an interaction between the BLA and ventral subiculum in determining the control over behavior by a conditioned reinforcer and its potentiation by increasing dopaminergic activity in the NAcc shell and this interaction may be subserved by convergent projections onto NAcc shell neurons. (Figure reproduced from Everitt *et al.*, 1999).

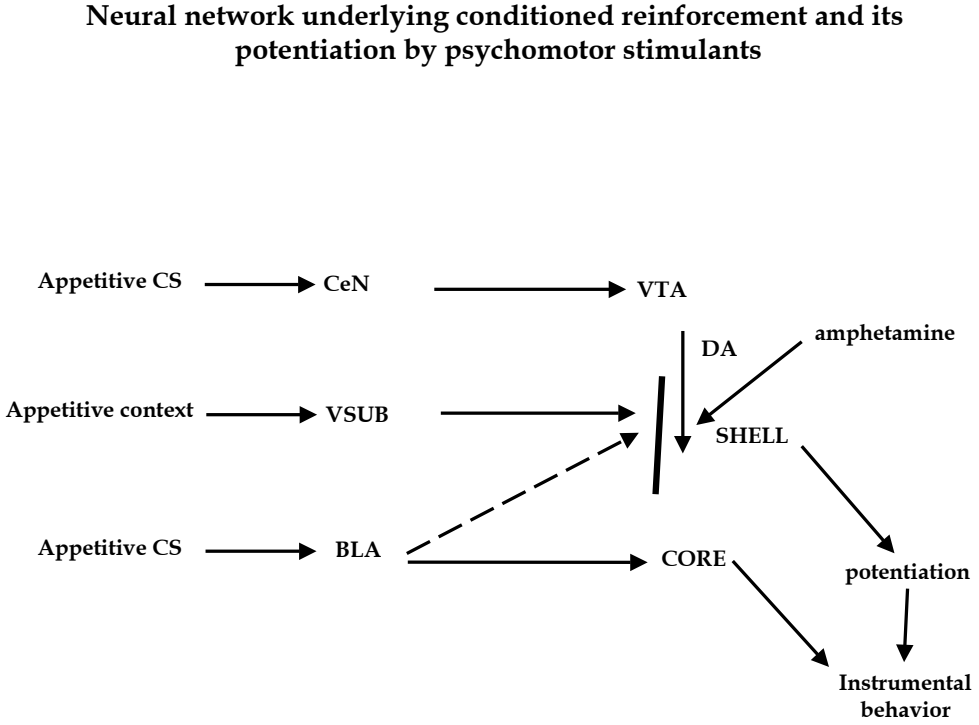

Figure 6
Simple associative representations. Plastic synapses change their weight on the basis of locally available information, requiring convergence of information about the representations to be associated.

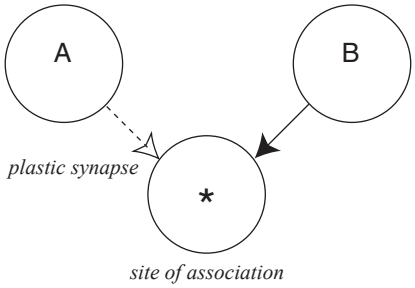

Figure 7
From a theoretical perspective pavlovian conditioning has the potential to create associations between a conditioned stimulus (CS) and representations of the unconditioned stimulus (US), central states such as fear, and unconditioned responses. Only a single response is shown; distinctions between different kinds of response are discussed in the text and shown in later figures. Bidirectional communication also allows representations to be associated in "third-party" sites (E). (Damasio *et al.*, 1993; Fuster, 1995) Note that lesions of such a site might prevent conditioning without impairing any form of unconditioned response, as would selectively *disconnecting* the CS from a representation involved in responding.

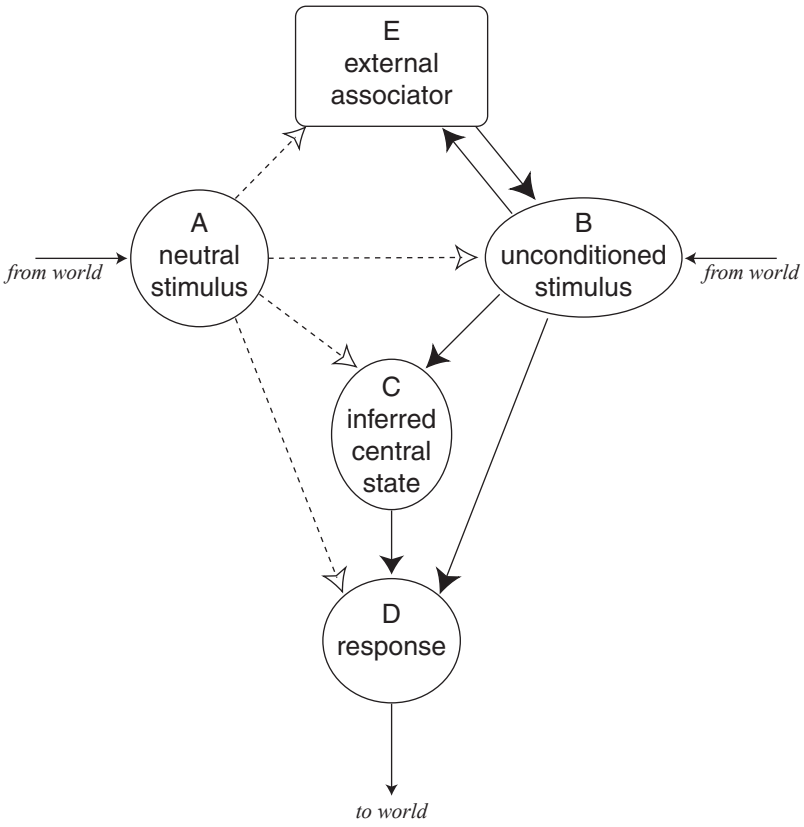

Figure 8
Conditioning to affective states leaves the response independent of the current value of the US. The CS associates with the affective state elicited by the US during conditioning, but if the US subsequently alters its value, the conditioned response (CR) will not alter.

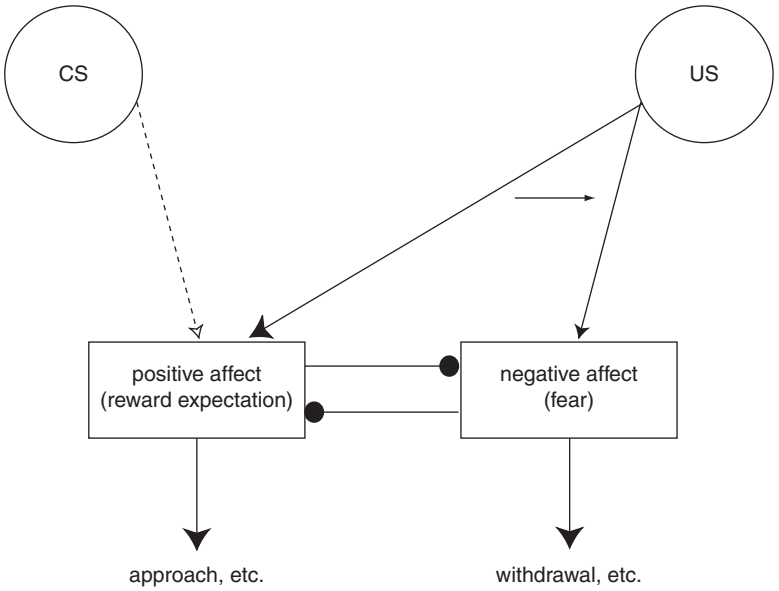

Figure 9

Schematic of representations that may be involved in pavlovian conditioning, emphasizing the hypothesized role of amygdaloid subregions. The BLA is required for a CS to gain access to the current value of its specific US. In the figure, the CS has been associated with US_1, initially appetitive, while an unrelated US_2 maintains a separate value (connections not shown for clarity). As discussed in the text, the precise nature of the information encoded in the BLA is uncertain; here, it is illustrated as binding US-specific sensory information to an affective value. The BLA may use this information to control CeN function but also to modulate specific instrumental (choice) behaviour, as in conditioned reinforcement tasks; the nucleus accumbens is a key target of this information. In contrast, the CeN is required for CS-UR learning, particularly when the response involves modulation of hypothalamic and brainstem functions.